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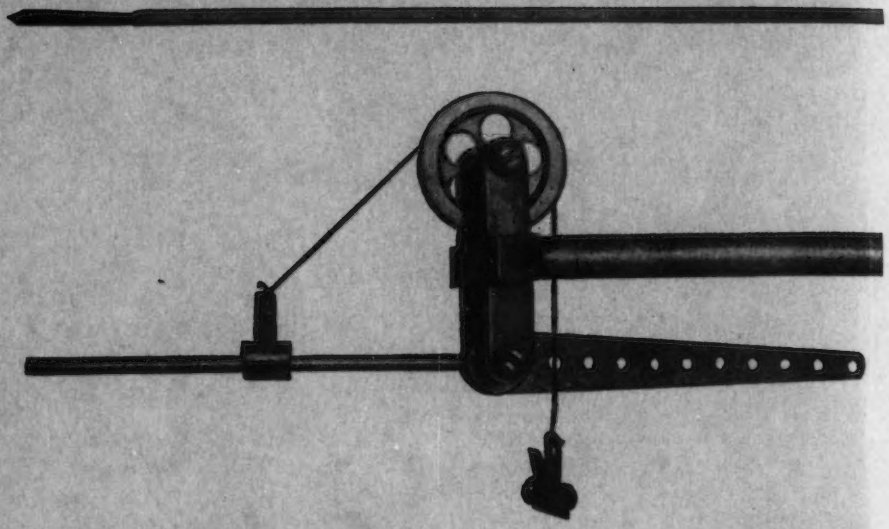

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STUDIES ON THE DOG'S CORTEX

I. The Sensori-Motor Areas[1]

S. HOWARD BARTLEY and EDWIN B. NEWMAN

From the University of Kansas and Bryn Mawr College

Received for publication April 14, 1931

The purpose of this paper is to report some of the behavior of the sensori-motor areas of the dog's cortex as revealed by recording the electrical phenomena from it. We have applied the term action current to the cortical event. However, the application of the term may not later seem advisable inasmuch as it does not pertain to linear propagation in a limited number of long fibres as it does in dealing with peripheral nerve trunks, but labels the activity that gives rise to disturbances in one's recording apparatus when the electrodes are placed anywhere on the cortex. Obviously the picture gained is gross compared with the measurements made by Gasser and Erlanger (1922 and later) at the periphery, but our work was undertaken in the belief that a gross picture to begin with would prove enlightening.

At this time we are reporting upon the sensori-motor areas in such a manner as to prepare the way for a fuller treatment of the whole cortex by the senior author. The records presented are from the sigmoid, cruciate, coronal, and the anterior ectosylvian gyri. In calling these the sensori-motor region, it is important to note several details of the interpretative history of their function. Fritsch and Hitzig were the first to directly investigate this area. As an outcome they clearly elicited bodily movements by faradic stimulation, especially of the sigmoid gyrus, and assigned motor functions to the latter, hence the *motor area*. Ferrier (1876) later showed that excitable points most surely exist not only in the motor area but also in the coronal, ectsylvian, and suprasylvian gyri. The latter gyrus came to be thought of as the visuo-praxic area on account of its adjacency to the visual area and on account of the movements elicited

[1] The work was done in the Psychology Laboratory of the University of Kansas.

being those of the eye. Munk's experiments indicated to him that the coronal gyrus was the center for sensory representation of the fore limb. A similar representation of the hind limb was found in the postsigmoid gyrus. In further experimentation by a different method, Dusser de Barenne (1916) (1924) on both cats and monkeys demonstrated sensory disturbances in dealing with these areas previously looked upon as motor centers. In other words, roughly the same limitations in cortical extent attended de Barenne's elicitation of sensory disturbances, as did faradic excitation in producing motor phenomena by such men as Fritsch and Hitzig and Ferrier. Hence we have this part of the cortex recognized as a sensori-motor area. Whether the area is sensory *and* motor or whether sensation and movement are only phenomenologically different is a worthwhile problem. It would seem that they must both obey the same laws of dynamics.

PROCEDURE. A few details of operative procedure and experimentation will be given first to make the records intelligible. Throughout our work the purpose has been to preserve as nearly as possible the intactness of the animal and to select individuals that were in good health and quite responsive. Exceptions to our intentions have occurred and the outcome in such cases has been used for purposes of comparison.

Ether was used as a general anesthetic. Along with it, a local injection of novocaine and adrenaline chloride mixed 2:1 was copiously applied to the tissues. The amount used seemed to be effective for both the operation and the recording period which began one or two hours later.

The desired portions of the skull were removed by first cutting out a small triangular section of bone with a rotary saw, and further by either end-cutting or side-cutting nippers. Nearly all of the potential operative hemorrhage threatened at the edges of the bone, and surgical wax (a mixture of bee's wax and mineral oil) was successfully applied. With caution, little hemorrhage ever occurred anywhere else.

With the operation completed, the electrode anchor was immediately located and fastened to the cranium, and artificial moisture and temperature supplied to the exposed brain from a thermally controlled source of Ringer's solution.[2] The electrode anchor, whose broad flat under-surface lay directly on the meninges was simply a metal clamp on the edge of the bone, and served in most of the recording as one of the leading-off electrodes. On the upper surface was a vertical post onto which the electrode holder was attached in any one of a variety of angles or altitudes. We employed several forms of electrodes and holders from time to time.

The most used form was a horizontal flat Bakelite bar on which two other flat pieces of the same material were mounted in rockers. Through the

[2] Taft, Robert. 1930. A convenient system of water thermostats for laboratory instruction. Journ. Chem. Educ., vii, 2053.

latter pieces the silver wires of the electrodes extended. The angle of the pieces was adjusted by a screw on the one end and an opposing compression spring on the other. With such an arrangement, the electrode tips could be located at the desired place on the brain and the pressure adjusted by the screw releasing the spring for action, the latter providing contact of the tips at all times during the usual brain volume changes. The electrode tips were never punctiform in nature even though small, for they were embedded in a matrix (sometimes of collodion) flush with its surface.

At times, four electrode tips were embedded in a common matrix but often in separate ones. With the latter arrangement, each of the four tips could be widely separated upon the brain. With the anchor as described above, used as a "neutral" or common lead, the circuit could be varied so as to include any one of the four tips, by means of a sliding switch. In this way records from diverse parts of the brain were gained without any adjustments necessary at the brain's surface. Quick shifts could be made in recording while the animal repeated or continued in its movements. Such procedure was our nearest approach to simultaneous recording from several points.

A wide variety of checks was made upon the recording system which consisted of a five stage audio frequency amplifier and a Westinghouse oscillograph. The conditions were ascertained which would produce artifactual excursions in the photographed records and were then avoided. A loud speaker was used in the output circuit of the amplifier so that we could investigate any suspicious new noise incidentally arising as we went along. This prevented a number of artifacts eluding us. To lessen the interference of the A.C. lighting circuit, the animal and the first three stages of the amplifier were housed in a shielded room of metal so constructed as to minimize acoustic disturbances as well. Lighting within the room was provided by storage batteries.

Records. The records included in this paper were those taken from the sigmoid, cruciate, coronal, and anterior ectosylvian gyri under the following sets of conditions: 1, dog quiet and no particular stimulation; 2, dog passive to experimental motion of its head; 3, dog passive to experimental motion of its limbs; 4, dog passive to pain stimulation; 5, dog wincing or slightly squirming to pain; 6, limb movements to experimental motion of its body; 7, gross movements to pain; 8, gross movements to experimental motion of its body; 9, head movements to pain stimulation; 10, head movements to experimental motion of body; 11, spontaneous movements of the animal.

Each of these sets of conditions has been termed a behavior situation. In the first one, the animal made no observable movements, and the room was quiet and almost always dimly lighted. With such conditions we should expect to have as little activity in the cortex as at any conceivable

time with an intact animal. It was found, however, that the records were not all patternless. In the second behavior situation, the animal remained virtually passive to movement of its head from side to side by the experimenter, while in the third it did not noticeably resist motion of one of its limbs up and down while it protruded through the slot in the canvas-topped table upon which the dog rested. In behavior situation four, the animal

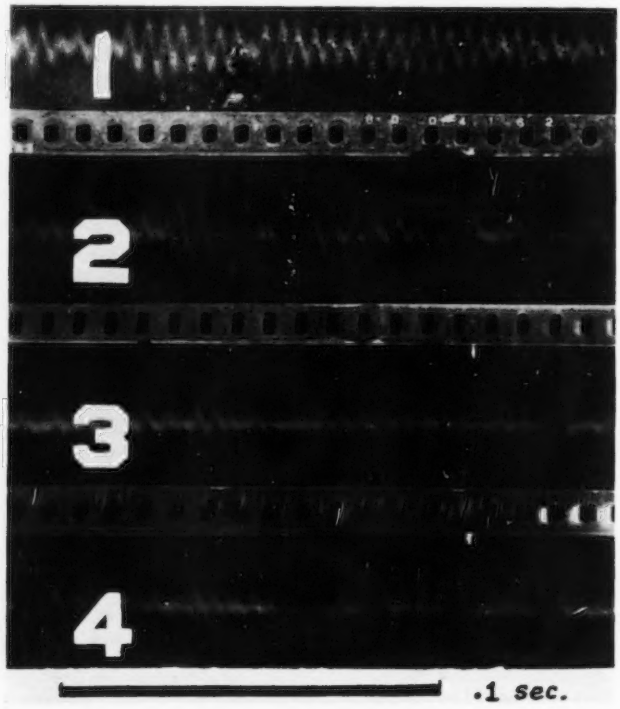

Fig. 1. This figure shows 4 records with intermittent patterns. Records 3 and 4 demonstrate some of the features of volley intervals. Records 1 and 2 are cases of longer trains of waves. Time shown in 0.1 second.

failed to respond overtly to the pinching of its tail. In situation five it squirmed or winced to the same stimulation and in situation seven it made gross bodily movements as a result. In situation six, the animal's body was rolled slowly back and forth on the table top, eliciting active movements as in walking. When the movements became those of a struggle as if to gain balance or release, the records were put into category eight. Class nine includes records taken when the animal shifted its head from

side to side in response to pinching its tail, and ten, those records when such movements resulted from its body being experimentally rolled as in situation eight. A few times when the experimenter was not stimulating the animal, struggling movements occurred. Records on these occasions were put into class eleven. It is obvious that none of the situations are precisely controlled. They are, nevertheless different one from another, and in that sense serve to show something of the functions of the respective gyri in a variety of conditions.

The records obtained were classified both as to amplitude and as to variation in pattern. The former classification is left till a survey of the whole cortex is given. In dealing with pattern, only the grosser aspects are considered. Little can be said about the finer details for no claim is made for absolute fidelity of reproduction in the amplifier, although it is possibly as good as many in use elsewhere. Since this is true, figure 1

TABLE 1

GYRUS	NEGATIVE	INTERMITTENT				EXTENDED		TOTAL
		Short		Long				
		Reg.	Irreg.	Reg.	Irreg.	Reg.	Irreg.	
	Z	A	B	C	D	E	F	
Ant.Sig.	2	8			2	1	5	18
Lat.Sig.	6	2	1	3	6		7	25
Post Sig.	2	2					7	11
Cruciate		1					1	2
Coronal	2		1	1	9	4	8	25
A.E. Syl.					1			1
Total	12	13	2	4	18	5	28	82

contains all of the records that need be shown. In it a partial account of the range in pattern obtained from the gyri is displayed.

The broadest classification in pattern made in the records is that of extension *versus* intermittency. Extended patterns are divided into those which are relatively irregular or complicated and those which are regular. The first order of subdivision in the records with intermittent patterns is that of shortness *versus* length. Both of these categories were further divided into regular and irregular groups. Thus, in all, the records fell into seven groups, A, B, C, D, E, F, and Z. The last group contains the records that showed so little disturbance as to be negative or equivocal.

The data obtained have been put into the form of two tables. Table 1 shows the number of records of each kind obtained from each gyrus, whereas table 2 takes into account the stimulation and the animal's behavior.

It can be seen that out of 82 records, 12 of them were of type Z, or

approximately 1 out of 7. If one considers the aspect of regularity, 22 out of 70 positive records are regular, or roughly 1 out of 3. The chances are seen to be about even, under our conditions, for obtaining extended patterns rather than short volleys. Of the intermittent patterns, about 3 out of 7 are classed as short. Also the short patterns were often quite regular in form. Thirteen cases out of 15 of the short were regular, while

TABLE 2

SIT.	A.SIG.	L.SIG.	P.SIG.	CRUC.	CORO.	A.E. SYL.
1	1F 2Z	2F 1Z			1E 2F	
2	1E 2A	4Z	1F 1Z		2F 2D	
3	1D 1F	1C 1F	2F	1A 1F	1Z 1C	
4		1B			1B	
5	1F	1F			1F	
6	2A	1Z	1A			
7	1F				2E	
8	2A 1F 1D	6D 3F 2C 2A	3F 1A		1D 3F	
9			1F		1E	1D
10			1Z		1Z	
11	2A				6D	

18 cases out of 22 were irregular for the long intermittent patterns, 28 cases out of 33 for the extended patterns.

From this it can be said that each gyrus behaved in diverse ways under different conditions. This variability of a point might be expected but would not be the case if one were to hold that any limited area when involved always makes the same contribution to the total activity, and that total activity is but the aggregation of one set of areas at one time and another set at another. It might be stated in this connection that large electrodes gave us records that were little if any more complicated than

did electrodes one-twelfth their size. The latter covered about one five-thousandth of the cortical surface.

Table 2 summarizes the data in relation to stimulation and the animal's behavior in keeping with the classification previously mentioned. This, of course, further subdivides the data so that the records dealt with for any one specified set of conditions are still fewer in number than if gyral positions alone were considered. There are enough cases for each behavior situation to show that a cortical point varies greatly in its function with the animal doing any one given thing and with one kind of stimulation. This has been shown in a simple algebraic way by Brown and Sherrington (1912) and has been referred to as inhibition and summation. Our data show that more conditions than those existing in a limited area must be taken into account before strict prediction would occur. The variability of the activity of any one point is to be understood in terms of what the whole system is doing. Thus the farther down into units the subdivision goes, the more unpredictable will be the behavior of that unit when considered alone, while it really is a part of a variable system.

Another feature of the records seen in either table is the lack of type A cases among the 25 (positive and negative) obtained from the coronal gyrus, and the occurrence of but 1 type B. Considering the rest of the gyri, there were 14 A records out of a possible 57. The greater proportion of the A records were taken from the anterior sigmoid gyrus and were fairly well distributed among the behavior situations. One-half of the positive records from this gyrus were of type A. This type of record presents intermittent short and regular patterns, whereas the majority of the records from the other gyri present long intermittent patterns or extended ones.

Type A by its nature brings us to a consideration of the time interval between the major patterns. It is in this kind of records that such measurements can best be made. Throughout our work records were not put into the intermittent class unless the trains of waves on them lasted for less than approximately one-half second. In some of the long intermittent records and some of the extended ones nodes followed each other at about the same intervals as the volleys themselves when isolated. We have found the frequency range of the volleys is 7 to 20 per second, with a majority of the instances occurring within a range of 9 to 12 per second. These are very near the values found by other investigators for the tremor rate in human skeletal muscle and for modulations in certain action current patterns.

Athanasiu (1923) found groupings of the impulses in his neuromyograms coming at about 10 per second; Hill (1921) using a tambour and hot-wire sphygmograph and recording with a string galvanometer presented records with the slowest variations at about 9 to 19 per second. Travis and

Hunter (1927) reported a modulation of from 6 to 12 per second in the higher frequencies of their neuromyograms.

Contrasting with these longer intervals, we have within our volleys the much higher rates which seem to be possible in both muscle and nerve. Cobb and Forbes (1923) for example, with electromyograms with a frequency as high as 66 per second, and Hill (1921) with the technique mentioned above demonstrated a frequency of fifty per second. The maximum frequency theoretically possible in nerve trunks in cases of voluntary muscular contraction has been shown to be 300 or more per second (Forbes, Ray and Griffith, 1923). The minor fluctuations found in some of our records approach this value. The closely corresponding rates in muscle and in the cortex to be found in our volley intervals suggest that possibly the same thing has been measured both at the periphery and in the cortex.

SUMMARY

In this paper we have briefly reported some of the findings from the dog's cortex using vacuum tube amplification and a Westinghouse oscillograph. In the study, variety both in stimulation and behavior of the animal were taken into account together.

The similarity between volley intervals in the records and the rate of tremor of skeletal muscle found by others was pointed out.

The outcome of the work tended to show that the behavior of any cortical point could only be made completely intelligible in terms of the behavior of the whole cortex.

BIBLIOGRAPHY

ATHANASIU, J. 1923. Journ. de Phys. et de Path. Gen., 21.
BROWN, T. G. AND C. S. SHERRINGTON. 1912. Proc. Roy. Soc., lxxxvB, 250.
COBB, S. AND A. FORBES. 1923. This Journal, lxv, 234.
DUSSER DE BARENNE, J. G. 1916. Quart. Journ. Exp. Physiol., ix, 355.
1924. Proc. Roy. Soc., xcviB, 272.
FERRIER, D. 1876. Functions of the brain. New York.
FORBES, A., L. H. RAY AND F. R. GRIFFITH. 1923. This Journal, lxvi, 553.
GASSER, H. S. AND J. ERLANGER. 1922. This Journal, lxii, 496.
HILL, A. V. 1921. Journ. Physiol., lv, Physiol. Soc., xiv.
TRAVIS, L. E. AND T. A. HUNTER. 1927. This Journal, lxxxi, 355.

OBSERVATIONS ON ADRENALECTOMY IN MARSUPIAL, HIBERNATING AND HIGHER MAMMALIAN TYPES

S. W. BRITTON[1]

From the Physiological Laboratory of the University of Virginia Medical School

Received for publication June 23, 1931

Preparatory to giving a report on various effects which are produced by the Swingle-Pfiffner (1931) cortico-adrenal extract, it seemed advisable to present some observations which have been made on representative types of adrenalectomized animals in this laboratory during the past few years. Hitherto most of these results have been given only brief reference elsewhere (Britton, 1930).

Adrenal excision in cats and rats. Normal survival periods and post-mortem conditions which were observed in bilaterally adrenalectomized cats are given in table 1. Removal of the adrenals was carried out in all 20 cases at one operation. From 10 to 15 minutes were usually taken to excise both glands *via* the anterior abdominal route. There appeared to be no advantage in removing the adrenals in two stages, as practised by most observers. In six cases of two-stage operated animals, the periods of survival were about a week or less.

If healthy, selected animals are used, the life-span of adrenalectomized cats appears generally to range between 5 and 10 days. In the 20 cases given in table 1 the range is between 3 and 11 days; the average is $7\frac{1}{2}$ days.

In a number of cases the blood sugar and non-protein nitrogen levels in adrenalectomized animals have been followed from day to day. The sugar values in cats fluctuated usually between 70 and 90 mgm. per cent for some days after operation, and no significant fall was observed until approximately the time that symptoms of adrenal insufficiency began to appear, or slightly earlier. In all cases the blood sugar in cats which showed slight weakness of the hind limbs, in walking, was found to be below the normal range noted above, for the analytical method used (Folin and Malmros, 1929). In table 2 it will be observed that in most instances the values are under 60 mgm., and in nearly half the cases they are below 50 mgm. The range is from 25 to 67 mgm. in animals showing different degrees of adrenal insufficiency.

The blood non-protein nitrogen levels are in contrast abnormally increased. Although in most cases the range lies within 45 and 80 mgm.,

[1] Grateful acknowledgment is made of aid received in this investigation from the Grants-in-Aid Committee of the National Research Council.

there are a few animals in which over 100 mgm. per 100 cc. were observed. The non-protein nitrogen method of Folin and Svedburg (1930) was used. Tests made on the blood cell volumes also revealed much increased values over the normal, readings up to 50 and 65 per cent being sometimes observed.

For comparative purposes observations were also made on a series of 20 white rats. Fifteen died on different days after the adrenals were removed as follows: 1 on the 3rd day, 2 on the 6th, 2 on the 8th, 1 on the 10th, 4 on the 13th, 1 on the 15th, 2 on the 17th, and 2 on the 25th. Three were surviving at the end of 40 days, and 2 at the end of 60 days after operation.

Slowly-developing muscular weakness, and eventually severe convulsive seizures, almost invariably heralded the death of adrenalectomized rats. The blood sugar was found to be low, and the non-protein nitrogen and blood cell volume very high, in a few cases which were examined when symptoms of insufficiency appeared. Lesions in the gastric mucosa and the pancreas were also observed at autopsy. It is therefore apparent that rats are affected similarly as are cats (and dogs) following elimination of the adrenal glands.

In only a few cases in rats have we found cortico-adrenal accessory tissue. The long post-operative survival may obviously be due to such accessories. The resistance shown by rats to severe operative insult indicates, however, that these animals may possess an extraordinary body structure which is well adapted to withstand the loss of even specialized organs or tissues. The relatively slight or imperceptible effects which follow removal of the thyroid or the parathyroid glands, or portions of the kidney tissue, or the amputation of one or two limbs, are well-known examples of the resistant body mechanism which is shown by the white rat.

Exclusion of the adrenal glands in the opossum (Didelphys virginiana) and the squirrel (Sciurus carolinensis). Observations which were made on a series of adrenalectomized opossums are presented in table 3. Both glands were removed at one operation, by the anterior route. The results are not significantly different from those noted in the case of other animal types. It is clear that the opossum does not long survive the excision of both adrenal glands.

In a few cases in which analyses were made, opossums showing symptoms of adrenal insufficiency (which were similar to those seen in other types) possessed very low blood-sugar values.

In two cases, squirrels were bilaterally adrenalectomized by the one-stage operation. Both animals lived only one day. We have not been able to keep full-grown, trapped squirrels alive in the laboratory, however, for more than a few days.

Observations on adrenalectomized marmots (Arctomys monax). Observations which were made on adrenalectomized groundhogs (Britton,

1930a), operated on at one sitting, have been extended. The recent experiments fully confirm the previous results Observations on summer-operated and winter-operated groundhogs are compared in tables 4 and 5. Notably the survival periods are in significant contrast. In summer the range of the survival period is from $1\frac{1}{4}$ to 10 days; in winter, from 29 to 128 days. In 14 cases operated on in summer (table 4) the average survival is $4\frac{1}{2}$ days; in 10 cases adrenalectomized in winter (table 5) the average is slightly over 72 days. Very low blood-sugar values and high non-protein

TABLE 1

Observations on adrenalectomized cats; single-stage operation

CAT NUMBER	SEX	WEIGHT	DATE ADRENAL GLANDS EXCISED	SURVIVAL PERIOD AFTER OPERATION	AUTOPSY FINDINGS	
					Congestion of pancreas	Congestion of gastric mucous membranes
		kgm.		*days*		
146	F.	1.8	3/ 6/29	11	+++	*
147	M.	2.2	3/ 6/29	10	+++	*
148			3/27/29	7.5	+++	*
149	M.	2.2	3/28/29	3	+++	*
150	M.	2.0	12/10/29	10	++	*
151	F.	1.8	12/10/29	4.5	0	*
152	M.	2.1	2/ 6/30	4	++	++
153	M.	2.8	2/11/30	10	+++	*
154	M.	2.2	2/13/30	10	+++	0
157	F.	2.1	2/13/30	6	++	++
158	M.	2.8	2/18/30	9	+++	*
159	F.	2.5	2/18/20	8	++	++
359	M.	1.6	2/23/31	11	++	+++
360	M.	3.0	2/23/31	8	++	++
363	M.	1.8	2/24/31	6	++	++
323	F.	2.1	1/31/31	5	++	++
324	F.	2.4	1/31/31	6	++	++
339	M.	1.9	2/ 9/31	9	++	++
338	F.	1.4	2/ 9/31	6	+++	0
346	M.	1.3	2/11/31	9	++	++

* Gastric mucosa not examined.

nitrogen levels were found in a few cases in animals which showed symptoms of adrenal insufficiency.

Most of the winter-operated groundhogs died, it is interesting to observe, with the approach of warmer weather in the spring, about the time of their natural emergence from hibernation. Two died in March, and five early in April. Two others which succumbed late in February, in their cage on the roof, were apparently victims of a severely cold spell of weather.

Marmots which were kept in dens on the roof during the winter did not

actually hibernate, it should be said; they were rather semi-dormant, sometimes for several days at a time, and only occasionally appeared outside the dens to take food. The striking difference in the survival of sum-

TABLE 2

Blood sugar and non-protein nitrogen levels in animals (cats) showing symptoms of adrenal insufficiency

CAT NUMBER	BLOOD SUGAR	BLOOD NON-PROTEIN NITROGEN	CONDITION	CAT NUMBER	BLOOD SUGAR	BLOOD NON-PROTEIN NITROGEN	CONDITION
	mgm. per cent	*mgm. per cent*			*mgm. per cent*	*mgm. per cent*	
252	61		Weak	350	49	57	Weak
255	67		Weak	357	50		Prostrated
258	43		In convulsions	359	54	57	Weak
259	65		In convulsions	360	42	118	Weak
261	39		In convulsions	363	52	81	Prostrated
285	39	74	Weak	365	32	91	In convulsions
316	57	60	Very weak	366	41	100	Weak
316	47	56	In convulsions	375	25	76	In convulsions
320		69	Prostrated	380	52	123	Weak
323	56	78	Prostrated	381	59		Weak
338	54		Very weak	383	63	83	Weak
339	50	71	Prostrated	386	51	142	Weak
342	54		Very weak	388	52	45	Weak
344	51	76	Prostrated	391	33	45	In convulsions
346	43	59	In convulsions	395	32	53	Very weak

TABLE 3

Survival and other observations on adrenalectomized opossums (Didelphys virginiana)

ANIMAL NUMBER	SEX	DATE OF OPERATION	WEIGHT BEFORE OPERATION	WEIGHT AT DEATH	SURVIVAL AFTER OPERATION	AUTOPSY FINDINGS	
						Congestion of pancreas	Congestion of gastric mucosa
			kgm.	*kgm.*	*days*		
1	F.	2/27/30	1.5		1	0	0
2	M.	2/27/30	2.3		18	+++	+++
3	M.	11/ 6/29	2.5		2	*	*
4	M.	11/ 6/29	2.2		1½	0	0
5	M.	11/11/29	1.9	1.6	15	++	+++
6	M.	11/29/29	2.4		21½	*	*
7	M.	11/30/29	2.5	2.0	33	++	+++
8	F.	11/ 7/30	1.6	1.4	12	*	*
9	F.	11/ 7/30	1.9	1.8	2½	++	++

* Tissues not examined.

mer-operated and winter-operated groundhogs probably finds explanation in the correlated differences in metabolic activity.

TABLE 4

Observations on adrenalectomized marmots (Arctomys monax)

In practically all cases the operations were done in the *summer*.

ANIMAL NUMBER	SEX	DATE OF OPERATION	WEIGHT BEFORE OPERATION	WEIGHT AT DEATH	SURVIVAL PERIOD	PANCREATIC CONGESTION AT AUTOPSY	CONGESTION OF GASTRIC MUCOSA
			kgm.	*kgm.*	*days*		
91	M.	7/ 2/29	0.9	0.8	4	*	*
92	F.	7/ 2/29	2.0	1.9	2	*	*
94	M.	7/23/29	1.3		$1\frac{1}{4}$	*	*
95	F.	7/23/29	1.2		4	*	*
96	M.	8/ 1/29	2.9	2.8	3	*	*
116	F.	5/10/30	2.4	2.2	3	+++	+++
117	M.	5/10/30	2.0	1.2	5	+++	+++
120	M.	5/13/30	2.2	2.1	2	++	0
109	M.	4/13/30	1.6	1.4	6	++	+++
121	M.	5/16/30	1.5	1.3	$4\frac{3}{4}$	++	0
122	M.	6/ 7/30	2.2	1.9	6	0	0
104	F.	6/ 8/30	1.2	1.1	4	*	*
111	M.	6/ 8/30	1.9	1.6	10	+++	+++
118	M.	6/ 8/30	2.2	1.9	6	+++	0
123	F.	6/17/30	2.5	2.3	4	+++	+

* Pancreas and stomach not examined.

TABLE 5

Survival and other observations on adrenalectomized marmots (Arctomys monax)

The operations were carried out during the *winter*.

ANIMAL NUMBER	SEX	DATE OF OPERATION	WEIGHT BEFORE OPERATION	WEIGHT AT DEATH	SURVIVAL AFTER OPERATION	CONGESTION OF PANCREAS AT AUTOPSY	CONGESTION OF GASTRIC MUCOSA
			kgm.	*kgm.*	*days*		
101	M.	11/20/29	2.8	1.1	90	++	++
102	M.	12/ 7/29	2.2	1.8	128	*	*
103	M.	1/ 8/30	1.9	1.4	33	*	*
110	M.	1/11/30	2.3	1.8	93	*	*
112	M.	2/20/30	2.6	2.1	40	+++	+++
113	F.	3/19/30	2.6	2.0	35	+++	0
114	M.	3/19/30	2.2	1.6	29	+++	0
131	F.	11/ 5/30	2.7	1.4	118	+++	++
133	F.	12/ 8/30	2.3	1.4	86	++	++
134	F.	12/ 8/30	2.4	1.6	70	+++	++

* Tissues not examined.

SUMMARY

The results of adrenalectomy in the marmot and the opossum are not dissimilar from those observed in the cat. With the earliest appearance of symptoms of insufficiency, the blood sugar becomes significantly reduced and the blood non-protein nitrogen considerably increased. Muscular weakness in all the types studied is then followed by convulsions (commonly) and eventually death ensues .

Summer-operated groundhogs usually survive for 2 to 10 days following adrenal removal. Opossums may live for a few days to a few weeks after the operation.

Death from adrenal insufficiency is inevitable although long delayed in winter-operated groundhogs. Such animals survive throughout the normal period of hibernation in apparent perfect health, although in common with their unoperated fellows they do not actually hibernate while in captivity. In early spring, death occurs with typical symptoms of adrenal insufficiency. The extended survival through the winter is attributed to the lowered metabolic demands during this season.

Pancreatic and gastric mucosal lesions are common in groundhogs and opossums, similarly as in cats dying after exclusion of the adrenal glands.

BIBLIOGRAPHY

BRITTON, S. W. 1930. This Journal, xciv, 686; Physiol. Rev., x, 617.
BRITTON, S. W. AND H. SILVETTE. 1931a. Science, lxxiii, 322, 373.
1931b. This Journal, xcvii, 507.
FOLIN, O. AND H. MALMROS. 1929. Journ. Biol. Chem., lxxxiii, 115.
FOLIN, O. AND A. SVEDBERG. 1930. Ibid., lxxxviii, 85.
SWINGLE, W. W. AND J. J. PFIFFNER. 1931. This Journal, xcvi, 153.

SOME EFFECTS OF CORTICO-ADRENAL EXTRACT AND OTHER SUBSTANCES ON ADRENALECTOMIZED ANIMALS[1]

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Within the past ten years there have probably been more attempts made by outstanding investigators to prepare an effective extract of the adrenal cortex than were ever before launched against a similar biological problem. Reports by a host of earlier investigators have been scattered through the press; unfortunately the ravages of time have not been charitably disposed in these cases. In a recent article (Britton, 1930) the many contributions on adrenal extracts have been given extended consideration.

The Hartman and Swingle-Pfiffner extracts. An advance which appears to be a consummation of the prolonged efforts of many investigators has been made by two schools of workers within the past year or so. Claims that potent aqueous extracts of the adrenal cortex have been prepared by Hartman and his collaborators at Buffalo (1930), and by Swingle and Pfiffner at Princeton (1931), have been given almost simultaneous publication. Earlier preliminary reports by both groups of workers have appeared. It is indicated from the experiments which are quoted in each case that preparations which possess considerable efficacy have at last been obtained.

In this laboratory we have prepared six batches of extract according to the technique devised by Hartman, and have tested the material a number of times on adrenalectomized cats. In two animals showing insufficiency symptoms we observed distinct signs of improvement following the intraperitoneal injection of 20 cc. of the extract. Another animal, cat 262, which was treated with 2 cc. daily of the Hartman extract, survived 31 days in good health; the extract was then withdrawn and the animal died overnight on the 41st day after the operation. At autopsy, however, a cortical accessory gland, of very small pea size, was found. While this would appear to invalidate the experiment, it may be said that in about twelve cases in which accessory cortical tissue has been found in 150 cats,

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life has been sustained after adrenal removal over two weeks in only two cases.

Apparently the cortico-adrenal hormone was contained at least in small amount in our extracts prepared according to the Hartman method. On paper this method appeared simple; it could be done very rapidly and was also inexpensive. The technical difficulties in preparation, however, particularly in the elimination of inert lipoid substances and of adrenalin, led us to postpone further work with the material. This step was taken particularly in view of consistently good results which we were concurrently able to secure with extracts prepared by the Swingle-Pfiffner method. We regret that a full report of the Hartman extraction method did not appear in the papers in which success with the extract was claimed. Details of the method have, however, appeared in print at the time of this writing (Hartman and Brownell, 1931). Perla and Gottesman (1931) state that "Cortin" protects adrenalectomized rats against histamine poisoning.

A more recent method of extraction is that reported briefly by Zwemer, Agate, and Schroeder (1931). Noteworthy results are said to have been obtained by the injection of this material (Zwemer and Sullivan, 1931). Only preliminary publications of the work are at hand, however, and further comment now is hardly justified.

Observations with the Swingle-Pfiffner extract. We have made and tested over sixty batches of cortico-adrenal extract made after the Swingle-Pfiffner process to date. At the kind invitation of Professor Swingle, two members of our staff were privileged to study intimately the technique of preparing the extract at the Princeton laboratories. Our first few batches of extract showed a low degree of potency; this was apparently because of failure at the abattoirs to remove the glands quickly enough from the cattle after slaughter. For several months now we have not failed to secure potent extracts.

Preliminary reports of our observations with the use of the Swingle-Pfiffner extract have been presented elsewhere (Britton and Silvette, 1931a, b). We have modified only slightly the method of preparation. The glands are exsected from the carcasses while still warm, and placed immediately in the freezing room at the abattoir. Shipment is made to the laboratory in carbon dioxide snow. To avoid the considerable labor of scraping out the medulla, we have from the beginning of our experiments used the whole adrenal glands in making our extracts. While still in the frozen state the glands are finely ground, and then placed in 95 per cent ethyl alcohol, in the proportion of 2.5 liters of alcohol to one kilo of glandular tissue. The mixture is stirred frequently for three days. The supernatant fluid is filtered off and concentrated *in vacuo* at 50–60 degrees to one-sixteenth the original volume. To the concentrate is added an equal volume of benzene, and the mixture is shaken and set aside in the ice-box for several hours. The benzene fraction is then siphoned off, and the alcoholic phase washed with fresh quantities of benzene until the washings are colorless. The tissue residue from the first alcoholic extraction is finely re-

ground, and then re-extracted with 80 per cent alcohol in the proportion of 2 liters of alcohol for each kilogram of whole glands originally used. After extraction for three days, with frequent stirring, the alcohol is filtered off, and concentrated in the same manner as in the first alcoholic fraction. Extraction with benzene is then carried out.

All the benzene washings from the first and second alcoholic fractions are then combined, the benzene is removed at a temperature of approximately 50 degrees, and the brown, sticky residue is thoroughly mixed with 1000–1500 cc. of acetone and placed in the ice-box over night. The acetone is then decanted off, and a fresh quantity added to the residue. This is allowed to stand in the ice-box, with frequent stirring, for several hours or overnight, and the acetone is then decanted. The residue is ground in a mortar with 100 cc. portions of acetone until the washings are colorless or only a pale yellow. The acetone washings are combined and distilled to dryness *in vacuo* at 40–45 degrees.

To the residue are added, in a 500 cc. separatory funnel, 30 cc. of petrolic ether, 74 cc. of 95 per cent alcohol, and finally 26 cc. of water. The funnel is carefully inverted so that the fluids are intimately mixed but still remain unemulsified. While still in the separatory funnel they are set aside in the ice-box for several hours, until partition between the petrolic ether and the 70 per cent alcohol phases is complete. The alcohol fraction is then run off, and 74 cc. of 95 per cent alcohol and 26 cc. of water are added, and the partition repeated.

Both alcoholic fractions are then combined and allowed to drip through a column of permutit (about 30 grams) which has been previously saturated with 95 per cent alcohol. The last traces of the active material are washed out from the permutit by 300 cc. of 95 per cent alcohol. The alcoholic solution is evaporated to dryness *in vacuo* at a temperature of 45 degrees. The quantity of distilled water necessary to make a final concentration of 40 grams of fresh whole gland per cubic centimeter of extract is added to the residue. After thorough mixing and maximum solution have taken place, the material is run through a Seitz filter into a sterile container, and it is then ready for use.

In the process of preparation, the adrenalin originally present in the tissue of the whole gland (present particularly in the medulla but partly also in the cortex, according to some observers) seems to a large extent to disappear. Small amounts only are left in the crude extract, the rest having been either destroyed by inadvertent oxidation or differentially partitioned between the various solvents employed. These traces are readily removed by a small quantity of permutit—we have used thirty grams for each five kilos of glands.

The final concentration of adrenalin present in our cortico-adrenal extracts, using this method with the whole glands, we have almost invariably found to be between 1:2,000,000 and 1:4,000,000, when tested by the surviving intestinal strip method. The products which we have secured for the past eight months, with these slight modifications of the Swingle-Pfiffner (1931) technique, are apparently as potent as any which have yet been reported.

It can be said positively that the extracts which we have made contain significant amounts of the cortico-adrenal hormone which is necessary for

the maintenance of life. The life-span of adrenalectomized cats is at least much prolonged, and apparently may be extended indefinitely, by the injection of suitable amounts of the material. In all cases under consideration except one (cat 279) we have used animals which were bilaterally adrenalectomized at one operation. When untreated, such animals in our experience (over 100 cases) survive usually for a week to ten days. There is a gradual weight loss; refusal of food is followed by symptoms of muscular weakness, complete prostration, and convulsive seizures. Death frequently occurs within 24 hours after the appearance of symptoms—almost invariably before the end of two days.

We have not given a great deal of our attention to observations on extending the life of adrenalectomized animals. At first, with small amounts only of the extract available, we tested the material on cats showing various degrees (symptoms) of adrenal insufficiency: there were found immediate and thoroughly convincing evidences of the efficacy of the extract, as shown in detail later. Thereafter we became interested in the possible blood-chemistry changes involved, and the influence of the substance on other bodily functions. Supplies of the extract were also given trial in clinical cases. Because of these considerations, as well as on account of the protracted and costly demands of the experiments, we have included in our series only a few cases in which the survival periods were prolonged beyond the normal limits. Brief protocols are given below.

Cat 257, female. Both adrenals removed October 31, 1930. Fasting blood sugar before operation, 80 mgm. per 100 cc.

November 2, in good condition; weight 1.50 k.

November 3, animal lethargic; extract commenced—1 cc. per kilo no. 4 intraperitoneally.

November 4 and 5, 1 cc. no. 4 per kilo; animal became much more active; weight 1.6 k.

November 6–13, cat in good condition; blood sugar 87 mgm.; 2 cc. extract no. 4 per kilo per day.

November 14, weight 1.66 k.; 2 cc. extract no. 4 per kilo.

November 15, appears well. Weight 1.6 k.; 2 cc. extract no. 4 per kilo per day.

November 16, fasting blood sugar 84 mgm. per 100 cc.

November 17–19, 4.5 cc. per kilo extract no. 4 per day.

November 20, 3 cc. per kilo extract no. 4 (all available); cat appeared well.

November 21, 9 a.m., found in very weak state; weight 1.55 k.; 9:25, severe convulsions; at 9:30, gave 16 cc. extract no. 11 intraperitoneally (10 cc. per kilo). At 11:30, blood sugar 71 mgm. per 100 cc.; cat much improved; ate large meal of salmon, washed itself. At 4:20 p.m., 6 cc. extract no. 11 (4 cc. per kilo); cat now very active, apparently normal.

November 22–23, in good condition; given 2 cc. per kilo extract no. 11 intraperitoneally.

November 24, cat slightly weak.

November 25, 8:30 a.m. to 11:45 a.m., cat in frequent convulsions; died at 11:50. Weight at death 1.4 k. At autopsy, abdominal viscera found to be congested; gastric and upper intestinal tract ulcerated; no accessory cortical tissues observed.

The potency of cortico-adrenal extract was very definite in the above experiment. The animal was kept in an apparently normal condition for 20 days after bilateral adrenalectomy; it gained in weight; its fasting blood sugar remained at normal levels throughout. At the end of this period the extract which was injected, in reduced amount, had been in the laboratory over one month, and had probably lost considerably in strength. The animal began to show symptoms of insufficiency, and convulsions set in. The condition rapidly cleared up, however, under dosage with freshly-made cortical extract. Later, when extract administration was stopped, the animal quickly began to fail, and died with typical insufficiency symptoms, 25 days after adrenal removal.

The observations on animal 279 are perhaps more positive. They are given below in brief.

Cat 279, female. November 26, right adrenal removed. December 9, 1930, left adrenal removed; weight 2.06 k.

December 10, treatment begun; 2 cc. per kilo extract no. 16 intraperitoneally.

December 11–16, 2 cc. extract no. 16 per kilo. Weight on December 16, 2.32 k. Cat in very vigorous condition.

December 17–20, 3 cc. extract no. 16 per kilo.

December 20–29, 3 cc. extract no. 23 per kilo. Animal appears normal, very active.

December 30–January 8, 1931, 3 cc. no. 24 per kilo. Weight on January 6, 2.52 k.

January 9, 1931, animal eating well, fat, active. Extract discontinued at this time.

January 13, weight 2.29 k. Cat lethargic; eating much less.

January 15, inactive; weight 2.26 k.

January 24, very slight weakness shown.

January 26, 8:30 a.m., cat found in convulsions; died at 8:45 a.m. with typical insufficiency symptoms. Weight at death 2.08 k. Autopsy—gastric mucosa slightly congested; otherwise negative.

After complete removal of the adrenals this animal survived with treatment for 48 days. Twenty-one days after operation it had gained 460 grams or 25 per cent in body weight. Following discontinuance of the extract there was a gradual loss of weight, and eventually the animal died with the usual symptoms of insufficiency.

In two other cases we kept animals alive after adrenalectomy, by giving extract treatment, for two weeks. Numerous observations on blood constituents were made on these animals. The injections of extract were withdrawn and death occurred within two days.

The restoration of adrenalectomized animals, showing symptoms of insufficiency, with cortico-adrenal extract. The rapid amelioration of even the severest symptoms which follow removal of the adrenal glands, by the Swingle-Pfiffner cortico-adrenal extract, is more astonishing than the development of the symptoms themselves. Whereas an animal, observed

about a week or ten days after operation, may obviously have been in a dying condition for many hours, indubitable signs of restoration to normal may appear as early as ten or twenty minutes after administration of the extract. Animals which have refused to take food for two or three days previously need no enticing, but have been observed to eat readily within two or three hours following extract injection.

In a cat showing severe prostration with adrenal insufficiency, six or eight days after excision of the glands, the typical course of recovery occurs as follows: Convulsions are suppressed within 5 to 10 minutes after the intraperitoneal injection of about 20 cc. of the extract, and in 10 or 20

TABLE 1

Observations on the resuscitation of adrenalectomized animals (cats) with extract of the adrenal cortex

CAT NUMBER	DATE	CONDITION BEFORE EXTRACT WAS GIVEN*	OBSERVATIONS
257	11/21/30	In convulsions	Improved in 30 minutes; walking about in 1 hour; ate 2 hours after injection
273	12/ 5/30	In convulsions	Improved 30 minutes after injection; in vigorous condition 6 hours later
273	12/ 7/30	In convulsions	Again rapidly resuscitated following administration of extract
273	12/10/30	Comatose	Sat up 15 minutes after injection; appeared normal 1 hour later
273	12/11/30	In convulsions	Recovery from extreme prostration in 4 hours
276	1/28/31	Comatose	Cat in vigorous condition 5 hours after injection
276	1/30/31	In convulsions	Appeared normal within 3 hours; ate salmon
316	1/29/31	In convulsions	Signs of recovery in 30 minutes; later ate salmon
338	2/13/31	In convulsions	Much improved in 1 hour; took food later
344	2/15/31	Prostrated	Walking about 15 minutes after injection
346	2/18/31	In convulsions	Improved within 30 minutes

* In all cases large amounts of extract, from 10 to 25 cc., were given (see later).

minutes a noticeable interest is shown by the animal in its surroundings. Before 30 minutes have passed it is usually sitting up, and within the hour it may be walking about normally, or at first with a somewhat rambling gait. During the second hour after injection a growing appreciation of a dish of food is evidenced, and eventually from 50 to 100 grams of meat may be taken. If more is allowed, vomiting may occur. More recently we have observed young animals take food ravenously as early as 45 minutes after the oral administration of extract. In table 1 are given several of our earlier observations of the recovery of animals, with cortico-adrenal extract, from different symptomatic degrees of insufficiency.

The decline of adrenalectomized animals and their recovery with extracts of the adrenal cortex may be in many respects compared to the decline of insulinized animals and their restoration with the adreno-medullary hormone. All phases in the latter case are commonly, to be sure, much quicker in action; in many instances the resuscitation from insulin shock

TABLE 2

Blood-chemical changes in adrenalectomized cats following injection of cortico-adrenal extract

In all cases the animals showed symptoms of insufficiency.

CAT NUMBER	DATE	AMOUNT EXTRACT GIVEN	EXTRACT NUMBER	BLOOD SUGAR			BLOOD NON-PROTEIN NITROGEN		TIME BETWEEN INITIAL AND FINAL BLOOD SAMPLES
				Before injection	After injection	Increase in blood sugar	Before injection	After injection	
		cc. per kgm.		*mgm. per cent*	*mgm. per cent*	*per cent*	*mgm. per cent*	*mgm. per cent*	*hours*
255	10/28/30	4	2	67	94	40			3.0
285	3/ 3/31	9	38	39	100	156	74	80	18.5
321	2/ 4/31	8	28	69	80	16			3.5
323	2/ 5/31	6	29	56	68	21			8.0
339	2/16/31	10	29	50	61	22			4.5
346	2/18/31	10	32	43	80	86			4.0
350	3/ 3/31	19	36	49	135	176	57	62	12.0
363	3/ 2/31	17	35	41	52	21			12.0
365	3/ 2/31	8	36	32	79	147			13.5
366	3/ 3/31	11	38	41	147	258	100	88	11.0
374	3/ 9/31	5	40	34	44	30			1.5
375	3/ 9/31	5	40	34	44	30			1.0
375	3/10/31	10	40	40	100	150			13.0
375	3/12/31	10	40	27	37	37			5.0
386	3/19/31	30*	42	65	87	34	147	44	24.0
388	3/29/31	10*	47	52	66	37	45	48	18.0
388	4/ 2/31	20*	44	57	92	61	50	24	2.5
389	4/10/31	20	48	54	98	66	41	27	25.0
398	4/10/31	20	48	60	84	40	51	34	8.5
444	5/18/31	20*	63	47	159	217			7.0
444	5/25/31	20*	65	43	147	241			7.5

* Extract given by mouth; all other injections given intraperitoneally.

with adrenalin is nevertheless protracted for a period approximating that taken by cortical extract to relieve adrenal insufficiency.

Changes in heart rate and body temperature. At the time of the first appearance of symptoms in adrenalectomized animals, the heart rate and also the body temperature have been found to be somewhat augmented. Later, subnormal levels have been noted, in common with other observers.

In the normal cat the resting heart rate is about 120 per minute, and the rectal temperature is about 38°. In adrenalectomized animals showing symptoms of insufficiency, changes toward or above the normal have been found in several cases following the administration of cortico-adrenal extract, as follows: heart rates, 124–160, 128–144, 80–110, 100–232, 90–180, 128–172; deep rectal temperatures, 37.6–38.9, 33.1–36.1, 35.3–38.0, 35.5–39.1, 36.5–38.8. That these changes are related to increased metabolic activities, such as have been observed recently (Swingle and Pfiffner, 1931), appears undoubted.

In the normal animal and in man we have observed no significant cardiac acceleration after cortico-adrenal extract administration. The quick changes which we have sometimes observed, it should be said, may be attributed to the presence of adrenalin in the extract.

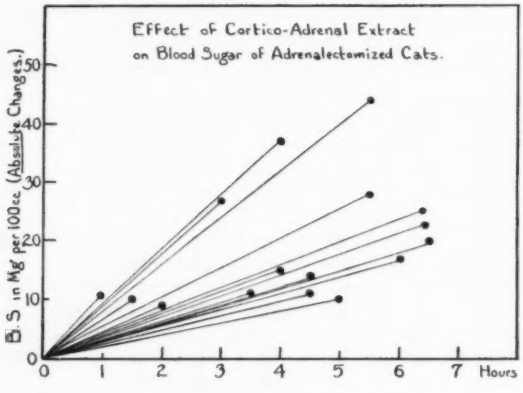

Fig. 1

The effects of cortico-adrenal extract on blood sugar and non-protein nitrogen. The occurrence of low blood sugar and high non-protein nitrogen levels in adrenalectomized animals has been repeatedly noted (Britton, 1930). The hypotheses that death following adrenal removal might be due to profound interference with carbohydrate metabolism (hence the marked hypoglycemia), or to a general intoxication (as with unremovable products of protein metabolism), led us to investigate the effects of cortico-adrenal extract on the connected blood-chemical conditions in animals showing symptoms of deficiency.

In summary in table 2 are given the changes which have been observed in blood sugar and non-protein nitrogen levels in a series of over twenty cases. The initial glycemic percentages before the injection of extract, it may be noted, are considerably below the normal levels, while the non-

protein nitrogen values are greatly augmented. Symptoms varying from slight weakness to severe prostration were present in all cases. The animals had been fasting for 24 to 48 hours before observations were carried out. Following cortical extract administration, there occurred noteworthy changes towards the normal in all but a few instances, in both sugar and non-protein nitrogen. The blood sugar method used was that of Folin and Malmros (1929), and the non-protein nitrogen method that of Folin and Svedberg (1930).

In five cases out of eight the blood non-protein nitrogen was significantly lowered, while in three cases there were negligibly small rises. The latter

TABLE 3

Data indicating that the blood sugar raising power of cortico-adrenal extract is proportional to the amount injected and the time during which it acts

CAT NUMBER	DATE	EXTRACT	EXTRACT NUMBER	BLOOD SUGAR			TIME BETWEEN BLOOD SAMPLES	*100 (q/w)t
				Initial	Final	Per cent rise		
		cc. per kgm.					*hours*	
316	1/28/31	5	27	57	74	30	6.0	1.0
	1/29/31	10	27	47	75	60	5.5	1.1
350	2/27/31	10	35	92	112	22	6.5	0.34
	3/ 1/31	12	35	81	95	17	4.5	0.32
365	3/ 3/31	6	38	79	103	30	6.5	0.91
	3/ 3/31	4	38	103	118	15	4.0	0.94
374	3/10/31	10	40	47	81	119	5.5	2.2
	3/10/31	10	40	47	100	171	8.0	2.1
444	5/18/31	20†	63	47	159	219	7.0	15.4
	5/25/31	20†	65	43	147	241	7.5	15.9

* Percentage rise in blood sugar, per cc. of extract injected (q) per kilo weight of animal (w) per hour (t).

† Extract given by mouth.

were practically within the limits of error of the method used. The tendency to approximate normal nitrogen levels was particularly observed when relatively large doses of the extract were given. In our earlier report sufficient consideration was not given to the latter point, and small changes only were observed.

Consistent increments in blood sugar followed in all cases the injection of cortico-adrenal extract (see fig. 1). In most cases the change was from the hypoglycemic to the normal level; in six experiments the sugar percentage rose to 100 mgm. per cent or considerably higher. The latter cases

represented increases above the initial levels of 100 to over 250 per cent. The hyperglycemia-producing effect on adrenalectomized animals of extract of the adrenal cortex is therefore apparent.

Blood sugar and non-protein nitrogen changes in normal animals. The effects of cortico-adrenal extract on blood sugar and non-protein nitrogen have been studied in a series of normal cats, and also in a few rats and rabbits. In all cases the results were similar to those observed in adrenalectomized animals. Table 4 presents the data derived from a series of normal cats.

Injections of the extract were given intraperitoneally. On both the blood sugar and non-protein nitrogen levels the effects were most marked at the fourth hour after injection. The larger amounts of extract which

TABLE 4

Changes in blood sugar and non-protein nitrogen produced in fasting, normal cats by cortico-adrenal extract

CAT NUMBER	AMOUNT EXTRACT GIVEN	BLOOD SUGAR				INCREASE IN BLOOD SUGAR	BLOOD NON-PROTEIN NITROGEN			
		Fasting level	After extract given				Fasting level	After extract given		
			2 hours	4 hours	5 hours			2 hours	4 hours	5 hours
	cc. per kgm.	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*
561	10	98	105	120	103	22	36	33	32	38
562	10	81	84	94	92	16	42			34
563	10	100	103	129	110	29	34		31	35
564	10	82	90	94	93	13				
565	20	100	144	171	141	71	37	34	29	35
566	20	102	116	135	109	32	39	36	27	31
567	20	101	118	150	117	49	36	35	30	31

were used produced the greater increases in blood sugar, and the great reductions in non-protein nitrogen. There were also observed slight diminutions in blood cell volume, but these were not outside the limits of error of the method.

It should be stated that young cats were much more responsive to the extract than older animals. The changes in rats and rabbits also were not as marked as in partly-grown cats and kittens. The fasting blood sugar and non-protein nitrogen levels are also affected in man as in animals, when the extract is given by intramuscular or intravenous injection (Flippin and Britton, 1931).

Influence on blood cell concentration. It is often difficult to secure even 0.1 cc. of blood peripherally from an adrenalectomized cat showing symptoms of insufficiency, and we have sometimes had to resort to cardiac puncture for samples. Within thirty minutes or so after the injection of cortical extract the blood flows readily from even a small ear vein, however,

and 1 cc. or more may be secured. Hematocrit determinations made on animals under extract treatment showed considerable shifts in blood concentration. In about twenty unoperated cats, it should be said, the readings of blood cell volume normally varied between 34 and 44 per cent. These values are much increased in adrenalectomized animals showing symptoms of insufficiency.

In several cases in which we made examination of adrenalectomized animals before and after extract injection the changes were as follows: Blood cell volume reduced from 49 to 40 per cent, 65 to 50, 49 to 43, 52 to 41, 54 to 47, 42 to 37, 38 to 33, and 36 to 27. These changes were observed a few hours after treatment, and the lower levels were often sustained for 12 to 48 hours.

Intraperitoneal injections of similar amounts of saline solution also produced diminutions from the high blood cell concentrations observed in adrenal insufficiency. In these cases, however, the shifts occurred rapidly, within a few minutes or an hour, and they were not maintained.

Proportional effects of cortico-adrenal extract. In cases in which the same batch of extract was used two or three times on the same adrenalectomized animal, it was observed that there was a direct effect on the blood-sugar level, according to the amount of extract given and the time through which it acted. A number of examples of this proportionality are given in table 3. An injection of 10 cc. per kilo produces approximately twice the percentage rise in blood sugar as does one of 5 cc. per kilo. It may be said that in general the blood-sugar-raising power of the extract is a direct function of the amount injected, and of the elapsed time.

For the same animal, and for the same or different days, amounts (absolute) of extract injected, and initial blood-sugar values, similar quotients are obtained, as shown in the last column of the table. From the different batches of extract used it is apparent that different figures are usually derived; the many and prolonged procedures involved in the present method of preparation would hardly allow, indeed, a uniform potency for the material.

Cortical extract effects compared with those produced by adrenalin. The possibility of the restorative effects and the blood-sugar changes being produced by adrenalin present in the extracts has been considered. A number of adrenalectomized cats with symptoms, and also normal animals, were injected with adrenalin in similar dilution and amount to that found in the cortical extracts used—i.e., between 1:2,000,000 and 1:4,000,000 parts of adrenalin, in amounts up to 10 or 20 cc. per kilo weight of cat. The results are shown briefly in table 5.

The recovery of enfeebled adrenalectomized animals followed within an hour or two after adrenalin administration, but the apparent good effect was not long maintained. The animals were not stimulated to take food,

as they were with cortico-adrenal extract. Vomiting followed, if somewhat larger doses of adrenalin were injected. The usual train of symptoms observed in adrenal insufficiency was apparently only slightly ameliorated by adrenalin which was given when the animals were weak or prostrated.

Such amounts and concentrations of adrenalin as were used, by intraperitoneal injection, produced only small increases in blood sugar in both adrenalectomized and normal animals. The changes were moreover quite transient—within an hour or so after injection, diminutions to the pre-injection levels were commonly observed.

TABLE 5

Adrenalin effects on cats showing symptoms of adrenal insufficiency

CAT NUMBER	DATE OBSERVED	CONDITION BEFORE ADRENALIN GIVEN	BLOOD SUGAR		RESULT
			Before adrenalin	After adrenalin	
			mgm. per cent	*mgm. per cent*	
205	12/14/39	Prostrated			Good temporary recovery; died within 12 hours
254	2/22/30	Prostrated	68	89	Apparently good recovery; died next day
259	2/25/30	Convulsions	55	63	Recovered temporarily; died 24 hours later
335	2/ 4/31	Convulsions			Rapid improvement; died next day
359	3/ 5/31	Weak	54	48*	Convulsions 6 hours after injection; died overnight
366	3/ 6/31	Slightly weak	57	62	Apparently restored
366	3/ 7/31	Weak			Temporary improvement
366	3/ 8/31	Very weak	52	57	Died overnight

* 5 hours after adrenalin; other samples taken approximately 1 hour after adrenalin given.

Between 10 and 20 cc. of 1:2,000,000 or 1:4,000,000 adrenalin, i.e., similar to the amounts and concentrations present in cortico-adrenal extract, were injected intraperitoneally.

In marked contrast to the foregoing is the gradual augmentation of the blood-sugar percentage following the injection of cortico-adrenal extract, to reach a maximum in about eight or ten hours. Single large doses of the material were observed to produce this effect. In 24 to 48 hours after injection, in animals in which both adrenal glands had been removed, the sugar level gradually declined, and the hypoglycemic condition again set in unless re-injections of the extract were given. In contrast also to the action of the cortical hormone is the merely temporary effect of adrenalin, as noted above, in resuscitating prostrated adrenalectomized animals (see table 5).

Our observations are not in agreement with the recent remarks of Swingle and Pfiffner (1931). The latter observers state that adrenalectomized animals with severe symptoms show no improvement in their condition and "derive no benefit from the injections" of adrenalin. At least a transient amelioration of the symptoms of adrenal insufficiency may be effected by the medullary hormone.

Extracts of testes, brain, liver, spleen, and heart muscle. Certain hormones and similar chemical agents which are normally found in large percentage in specific bodily organs may also be present in other variously-situated tissues of the body (e.g., insulin, adrenalin). The mesodermal origin of

TABLE 6

Effects of glucose solution on animals suffering from adrenal insufficiency

CAT NUMBER	DATE OBSERVED	CONDITION BEFORE GLUCOSE GIVEN*	BLOOD SUGAR		RESULT
			Before glucose	After glucose	
			mgm. per cent	*mgm. per cent*	
250	2/ 6/30	Weak	67	75	Died 9 hours after injection
273	12/12/30	Convulsions			Slight improvement; died 6 hours after glucose given
323	2/ 5/31	Prostrated			Temporary improvement; died next day
357	3/ 3/31	Very weak	50	107	Improved, but died 4 hours after injection
285	3/ 4/31	Weak			Died over night
350	3/ 4/31	Slight weakness			Animal died next day
381	3/18/31	Weak	59	250	Apparently restored; died 30 hours later
405	4/ 4/31	Prostrated			Apparent recovery; found dead next day

* Usually between 10 cc. and 20 cc. of 10 per cent or 20 per cent glucose solution were given intraperitoneally.

the gonads and their partly comparable cell-structure, and the high lipoidal content of the cerebral tissues, suggest correlations in activity with the cortex of the adrenal glands. The development of a method by which potent extracts of the adrenal cortex may be consistently produced has allowed the testing of extracts of these and other tissues.

Extracts of the testes, brain, liver, spleen, and heart muscle have been tested on cats showing different degrees of adrenal insufficiency. From 100 to 300 cc. of each extract were prepared, the same technique as that used for cortico-adrenal extract being employed in all cases. Large doses were given, and each extract was tested on three or more animals. In every case, however, the results were wholly negative: the symptoms

and blood-chemical conditions of adrenal insufficiency were not affected, and the post-operative survival periods were not extended. Similar negative results were observed in a series of cases in which pituitrin, ergotamine, and ephedrine solutions were tested.

Effects of glucose on adrenal insufficiency. Observation that noteworthy rises in blood sugar were correlated with recovery by cortico-adrenal extract from the severe prostration of adrenal insufficiency led to the consideration of glucose effects on the condition. Eight animals showing different degrees of insufficiency were injected with varying amounts and strengths of glucose solution, up to 25 cc. of a 20 per cent solution. In a few cases the blood-sugar levels were determined before injection and just before death of the animals, which usually occurred several hours after glucose treatment.

It will be observed from table 6 that only temporary improvement in condition followed the administration of glucose solution; commonly, indeed, death occurred within 24 hours. In the cases examined, the sugar values were nevertheless increased to within the normal or even hyperglycemic levels.

Insulin shock and cortical extract. Hypoglycemic and convulsive reactions which follow insulin overdosage appear to be scarcely affected when large doses of extract of the adrenal cortex are given intraperitoneally or intracardially. When the material is given as a preliminary measure, an hour or so before the administration of insulin, the action of the latter also appears to be relatively unaffected. The possibility that a protective effect against insulin shock may be secured by cortical extract injection some days previously to insulin injection was furthermore not sustained. The well known effect of adrenalin in alleviating the severe symptoms of insulin intoxication does not find a parallel in the action of the new cortico-adrenal hormone.

The biological assay of cortico-adrenal extract. Changes in the blood constituents of both adrenalectomized and normal animals such as reported herein suggest methods of biological assay of the extract. On normal animals a considerable amount of the material is necessary to bring about a notable increase in blood sugar, and no positive indication of the presence of the life-maintaining hormone is secured from such an experiment. Young animals apparently respond better than older ones. The restoration of animals showing symptoms of adrenal insufficiency, and the concurrent rise in blood sugar, have furnished indubitable evidence of hormonal potency in our experiments.

Adrenalectomized cats showing symptoms of deficiency are therefore suggested as very suitable test objects for cortico-adrenal extracts. We feel that there is still considerable variability in the strength of the extracts prepared according to the Swingle-Pfiffner technique. Five cubic centi-

meters per kilo of an ordinarily potent extract should nevertheless restore a prostrated, adrenalectomized cat to an apparently normal condition in five hours, with an accompanying blood sugar increase of 25 to 50 per cent.

The possible significance of blood-sugar changes produced by cortico-adrenal extract. The increases in available sugar in the blood which follow cortico-adrenal extract administration stimulate inquiry on many considerations. While the blood sugar in untreated adrenalectomized animals is very low, the blood volume is apparently much diminished; when the sugar level is augmented by extract injection, the circulating blood appears to be notably increased. Considerable percentage increases in blood sugar frequently take place. The *absolute* amount of sugar in the blood is, therefore, probably very greatly increased.

These changes are produced notwithstanding the great reduction in hepatic glycogen reserves which occurs in severe adrenal insufficiency. In the muscular tissues also the glycogen is notably decreased. It would seem from the present results that an important and necessary function of the cortico-adrenal hormone is therefore to provide the indispensable carbohydrate fuel for use by the muscular tissues, by making it available through the blood stream. Perhaps this is indicative of the primary action which is involved. The concurrent circulatory and temperature effects would appear to be general expressions such as are related to all body recovery processes. The non-protein nitrogen changes in the blood, which are relatively small in extent, are possibly also indirect actions of the hormone, brought about by enhanced renal secretion following circulatory improvement.

While the blood sugar increments might possibly be effected through glycogenolysis occurring in the liver, other sources should be considered. The very slow rise in the sugar level which follows extract injection does not, indeed, suggest the hepatic origin of the material. The formation of carbohydrate from blood lactates, or possibly from fatty acids or protein materials, should indeed be entertained. In a few preliminary experiments in which we have tested some of these possibilities, the results indicate that the blood lactates are much increased in cases of adrenal insufficiency, and that decreases are brought about, along with the increase in blood sugar, by cortico-adrenal extract administration. In some cases, too, the liver glycogen percentage is increased. These observations are at present being given further test.

Notes on dosage, and the possible presence of two hormones in the adrenal cortex. The necessity of administering large amounts of extract in order to bring about the resuscitation of adrenalectomized animals became apparent early in the work. The quantities used represented relatively huge amounts of cortical tissue—sometimes, indeed, two thousand times or more than the amount present in the normal cat. Swingle and Pfiffner, as

well as ourselves, often gave from 15 to over 30 cc. of the extract in order to bring about the restoration of animals with symptoms of insufficiency.

Such amounts of extract represent, it may be noted, up to 1,000 grams of adrenal cortex, given in one or more doses in the course of about twelve hours. And the normal cat of about two kilos in weight possesses only from two to three hundred milligrams of cortico-adrenal tissue altogether! That previous observers may not have secured noteworthy effects with their cortical extracts because of failure to employ it in sufficient, heroic dosage appears somewhat likely.

It is interesting to note, however, that these very large doses of extract have apparently no nocuous effects on small animals when given intraperitoneally. We have also observed it to be non-toxic when given by subcutaneous, intramuscular, intravenous or intracardial injection. The latter route is particularly effective in resuscitating comatose animals. We have frequently used the extract in doses of 20 to 30 cc. intravenously in man without ill effect (Flippin and Britton, 1931).

The yield of hormone per gram of tissue in the extract made by the present methods does not appear large. Extracts made according to the Swingle-Pfiffner technique do, however, contain readily demonstrable amounts of the potent factor present in the adrenal cortex. To only the casual observer the results of the biological method of proof of hormonal potency must appear convincing. Even when the final word of chemistry has been said on the matter, it hardly seems likely that the crucial test on the organism will have lost its force.

The objection of some observers to the term "hormone" in the present case thus does not appear valid. It may be suggested, indeed, that the possibility of the presence of at least two hormones in the cortico-adrenal tissues may be entertained. In the present series of papers the demonstration that profound effects are brought about by cortico-adrenal extract on such widely different activities as are represented by carbohydrate metabolism and sexual development (Corey and Britton, 1931) invites early consideration of this possible duality.

SUMMARY

The survival period of adrenalectomized cats may be prolonged and possibly indefinitely extended by the administration of extract of the adrenal cortex, which has been prepared in this laboratory according to the method of Swingle and Pfiffner. The repeated resuscitation of operated animals with cortico-adrenal extract, even when they show the severest symptoms of adrenal insufficiency, offers very convincing testimony of the potency of the material.

The restorative effects produced by cortico-adrenal extract on adrenalectomized animals are somewhat similar to those of adrenalin on insulin

shock, although they occur more slowly: the convulsions and weakness of adrenal insufficiency are abolished, the blood sugar is increased from hypoglycemic to normal or even hyperglycemic levels, the body temperature is raised, and the heart rate is increased; a ravenous desire for food may then be shown by the animal. This interesting parallelism in activity between the hormones of the cortical and medullary portions of the adrenal gland is discussed. The convulsions of insulin hypoglycemia are, however, not affected by cortico-adrenal extract.

The blood-sugar-raising ability of the extract has shown a definite proportionality in many cases: in general, it is a direct function of the amount of material injected and of the elapsed time.

Adrenalectomized cats showing symptoms of insufficiency are very suitable test objects for cortico-adrenal extracts. Five cubic centimeters per kilo of an ordinarily potent extract should restore a prostrated animal to a normal condition, with an accompanying blood sugar increase of 25 to 50 per cent, within five hours.

Significant diminutions in the non-protein nitrogen, and in the blood cell concentration, are also produced in cases of experimental adrenal insufficiency by the administration of cortico-adrenal extract.

Blood sugar and non-protein nitrogen changes are found in normal fasting rats, rabbits and cats, and also in man, following extract injection. The changes are not so great as in operated animals, however, and they are also less prolonged.

The effects of the extract on adrenalectomized animals are not due to the small amount of adrenalin it contains. When animals showing insufficiency symptoms are injected with adrenalin in similar dilution and amount to that found in cortico-adrenal extract, the restorative effects are merely temporary and death is postponed for a few hours only. The action of glucose solution is also only slightly palliative.

Extracts of the testes, brain, liver, spleen, and heart muscle, prepared in the same manner as cortico-adrenal extract, have been tested on animals showing different degrees of adrenal insufficiency. In all cases the results were negative. Pituitrin, ephedrine and ergotamine solutions were also without effect.

Relatively huge amounts of extract, representing in cortical tissue up to two or three thousand times the amount present in the normal cat, are often necessary to effect the recovery of prostrated, adrenalectomized animals. Low yields are apparently obtained by the present extraction methods.

Observation that animals from which all the adrenal tissue has been removed may be kept alive with extracts of the cortex indicate the dispensability but not the inutility of the adrenomedullary secretion.

The possible significance of the blood-sugar changes which are produced

by cortico-adrenal extract is discussed. It is suggested that the primary action of the (life-preserving) hormone of the adrenal cortex is concerned with the maintenance of normal carbohydrate balance in the body.

Consideration that cortico-adrenal extract maintains the life of adrenalectomized animals, and also markedly influences sexual maturity—two apparently widely-separated functions—suggests the presence in the material of two distinct hormones.

BIBLIOGRAPHY

Britton, S. W. 1930. Physiol. Rev., x, 617.

Britton, S. W. and H. Silvette. 1931a. Science, lxxiii, 322, 373.

1931b. This Journal, xcvii, 507.

Corey, E. L. and S. W. Britton. 1931. Science, lxxiv, 101.

Flippin, J. C. and S. W. Britton. 1931. Unpublished observations.

Folin, O. and H. Malmros. 1929. Journ. Biol. Chem., lxxxiii, 115.

Folin, O. and A. Svedberg. 1930. Ibid., lxxxviii, 85.

Hartman, F. A., K. A. Brownell and W. E. Hartman. 1930. This Journal, xcv, 670.

Hartman, F. A. and K. A. Brownell. 1931. Science, lxxiii, 620.

Perla, D. and J. M. Gottesman. 1931. Proc. Soc. Exp. Biol. Med., xxviii, 650.

Stewart, G. N. and J. M. Rogoff. 1929. This Journal, xci, 254.

Swingle, W. W. and J. J. Pfiffner. 1931. This Journal, xcvi, 153.

Zwemer, R. L., F. J. Agate and J. A. Schroeder. 1931. Proc. Soc. Exp. Biol. Med., xxviii, 721.

Zwemer, R. L. and R. C. Sullivan. 1931. Ibid., 723.

THE INDUCTION OF PRECOCIOUS SEXUAL MATURITY BY CORTICO-ADRENAL EXTRACT[1]

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Considerable evidence has accumulated over many decades that a close connection exists between cortico-adrenal activity and the functions of the sex glands. Although chiefly clinical, the observations have commonly shown a striking consistency, and an intimate physiological interrelationship is now considered by many to be well established. One text book (Macleod, 1930) brings forward a great deal of evidence under seven headings indicating that the adrenal cortex and the sexual organs are related.

As long ago as 1806 it was pointed out by Meckel that anatomical and certain pathological conditions suggested an intimate association of the adrenals and the gonads (paper by Nagle, 1836). In more recent years Bullock and Sequiera (1905) and Guthrie and Emery (1907) have related hypertrophy of the adrenal cortex to sexual precocity and to pregnancy. That growth and differentiation of the sex cells is dependent in some way on cortico-adrenal secretion has been advanced by Glynn (1912, 1921). A good deal of the later literature on the subject may be found in the papers by this author. Knowledge that both cortico-adrenal and gonadal tissues arise in the embryo from a common mesodermal primordium, and also that there is a striking similarity between the cells of the cortex and those of the corpus luteum, gives further weight to the above views. A report by Müller (1931) indicates, however, that adrenalin-free extracts of the adrenal cortex may produce regressive changes of the sexual apparatus in rats.

Feeding experiments which have been carried out by Hoskins and Hoskins (1916) and by McKinley and Fisher (1926) are in part corroborative. Jaffe and Marine (1923) have noted ovarian enlargement, affecting chiefly the interstitial cells, in rabbits which had been adrenalectomized and survived over thirty days. No changes were found in the testes.

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The length of the oestrous cycle in rats was unaffected after adrenal removal, according to Schiffer and Nice (1930). Recent reports (Papanicolaou, 1931) on changes produced in the adrenal cortex by injections of urine, obtained from both pregnant women and cattle, are suggestive.

MATERIALS AND METHODS. While the clinical literature is therefore definitely indicative on the subject, experimental data in confirmation of the gonad-adrenal hypothesis are meager and inadequate. The development within the past year or so of potent extracts of the adrenal cortex (Hartman and Brownell, 1930; Swingle and Pfiffner, 1931) have led, however, to a new era in adrenal physiology, and many indications of the profound effects of this material have already been reported (Hartman *et al.*, 1930; Swingle and Pfiffner, 1931a, b; Britton and Silvette, 1931a, b; Zwemer *et al.*, 1931a, b). During the past year we have prepared over sixty batches of cortico-adrenal extract in this laboratory, and have used it experimentally and also clinically (Britton and Silvette, 1931a, b). In view of the foregoing considerations, we have tested these extracts of the adrenal cortex, which were made according to the Swingle-Pfiffner technique, on immature male and female rats, with the possibility in mind of producing early sexual activity in such animals. A preliminary report (Corey and Britton, 1931) indicates that positive results have been forthcoming in these experiments.

Albino rats of Wistar strain were used throughout the investigation, the original stock entering the laboratory about six months previously. The animals were fed on a standard mixed meal diet. No deaths occurred among either the experimental or the control animals throughout the entire period of experimentation. Injections of extract were in all cases made intraperitoneally. The different batches of extract used were protein-free and sterile; they were also of proven potency, as demonstrated by their effectiveness in resuscitating prostrate adrenalectomized cats, and in maintaining them alive in good condition. Litter-mate controls were used in all cases; these were injected with similar amounts of normal saline solution. Furthermore, injections of similar quantities of adrenalin of like dilution to that found in the cortical extract—1:2,000,000—were also given in control experiments. The latter failed, it may be said, to elicit any maturation response.

RESULTS. No ill effects were observed on the administration of the Seitz-filtered cortico-adrenal extract, even in the case of 4-day-old rats. The animals appeared to be in normal good health throughout the period of injection. Nursing animals were frequently observed, indeed, to return to the mother within a few seconds after injection. All organs examined were fixed in Bouin's solution, and cleared in xylol. Identical treatment of all tissues was rigidly practised throughout.

Briefly, the chief observed results of injection of the extract were pre-

TABLE 1

LITTER NUMBER	NUMBER OF ANIMALS INJECTED	AGE WHEN KILLED	DURATION OF PERIOD OF INJECTION	DAILY DOSAGE	EQUIVALENT IN GLAND (CORTICAL) SUBSTANCE	OBSERVATIONS ON EXPERIMENTAL ANIMALS
		days			*grams*	
1	4	18	1 week—2 2 weeks—2	¼ cc.	7.5	Male and female organs immature
2	4	20	5 days	1 cc. per 10 gr. rat	30	Pituitary hypertrophy; slight if any change in gonads
3	5	20	3 days	2 cc. alternate days	60	All cases negative; pituitary not sectioned
4	4	20	1 week—2 2 weeks—2	2 cc.	60	Organs immature; pituitary not sectioned
5	4	25	1 week—2 2 weeks—2	½ cc.	15	Negative; pituitary not sectioned
6	6	28	2 weeks	1 cc. alternate days	30	Ovaries maturing; follicles large; uterine hypertrophy not observed
7	5	28	2 weeks	1 cc.	30	Markedly positive in female; follicles much enlarged; uterine hypertrophy; corpora lutea present; hemorrhages
8	4	32	1 week—2 2 weeks—2	¼ cc.	7.5	Results positive; corpora lutea; no marked change in male organs
9	4	33	1 week	4 cc.	120	Positive; corpora lutea present; no apparent change in males
10	4	33	5 days	2 cc.	60	Positive; pituitary hypertrophy; large follicles in ovary; no change in males
11	6	34	2 weeks	1 cc. alternate days	30	Ovaries showed definite maturation
12	4	35	1 week	1 cc.	30	Positive reaction; corpora lutea; spermatogenesis in males
13	7	37	2 weeks	1 cc. alternate days	30	Ovaries fully mature
14	2	42	1 week	1 cc.	30	Positive; corpora lutea; spermatogenesis in males*

Litters used, 14; experimental animals, 63.

* Corpora lutea were present in none of the control animals. At this stage (42 days), however, a small percentage (16 per cent) of the tubules of the controls contained spermatozoa.

mature maturation of the sex glands, marked uterine hypertrophy, and enlargement of the pituitary gland. Table 1 presents the essential data in tabular form.

The earliest response was seen at 20 days of age (litter 2). In this case pituitary hypertrophy was the only change that was observed on section of the tissues (figs. 11 and 12). No alteration in the gonads could be positively determined. Litters 3 and 4 are tabulated as negative; in these, sections of the hypophysis were not made, and no statement can therefore be made regarding them. No changes in the gonads were evident.

At 28 days of age (litter 6), a markedly positive response was evoked. Not only did the ovaries exhibit corpora lutea, but a considerable hypertrophy of the uterus had taken place (cf. figs. 3, 4, 5, and 6). From this age-period onward, maturation responses were elicited in all of the injected female animals. No striking response was observed in the males, however, until they had attained an age of 35 days. At this time the histological differences between the control and experimental tissues were clearly apparent (figs. 15 and 16). Changes in the testes are subsequently described in detail under separate heading. At 42 days of age some of the control males showed spermatozoa in the sectioned tubules, and experimentation on older animals was thus invalidated from the comparative standpoint.

Ovary and uterus. Sections of the ovary, photographs of the uteri, and cross-sectional microphotographs are shown in figures 1 to 10. Evidences of early maturation following the injection of cortico-adrenal extract were most striking in the female. The presence of corpora lutea in the ovary, and uterine hypertrophy, were considered particularly as indicative of sexual maturity.

The first positive results appeared in 28-day-old rats. In these the uterus was sometimes hypertrophied, and the ovaries showed many enlarged follicles. Occasionally corpora lutea were present. It seems possible that positive findings might appear somewhat earlier than this stage if

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Fig. 1. Section through the ovary of a 28-day-old control animal.

Fig. 2. Section through the ovary of its litter-mate which received 1 cc. of extract daily for 2 weeks (same magnification).

Fig. 3. Uterus of 28-day-old rat which received 1 cc. of extract daily for 2 weeks (about × 2).

Fig. 4. Uterus of its litter-mate control (about × 2).

Fig. 5. Uterus of 28-day-old rat which received 1 cc. of extract daily for 2 weeks (about × 2).

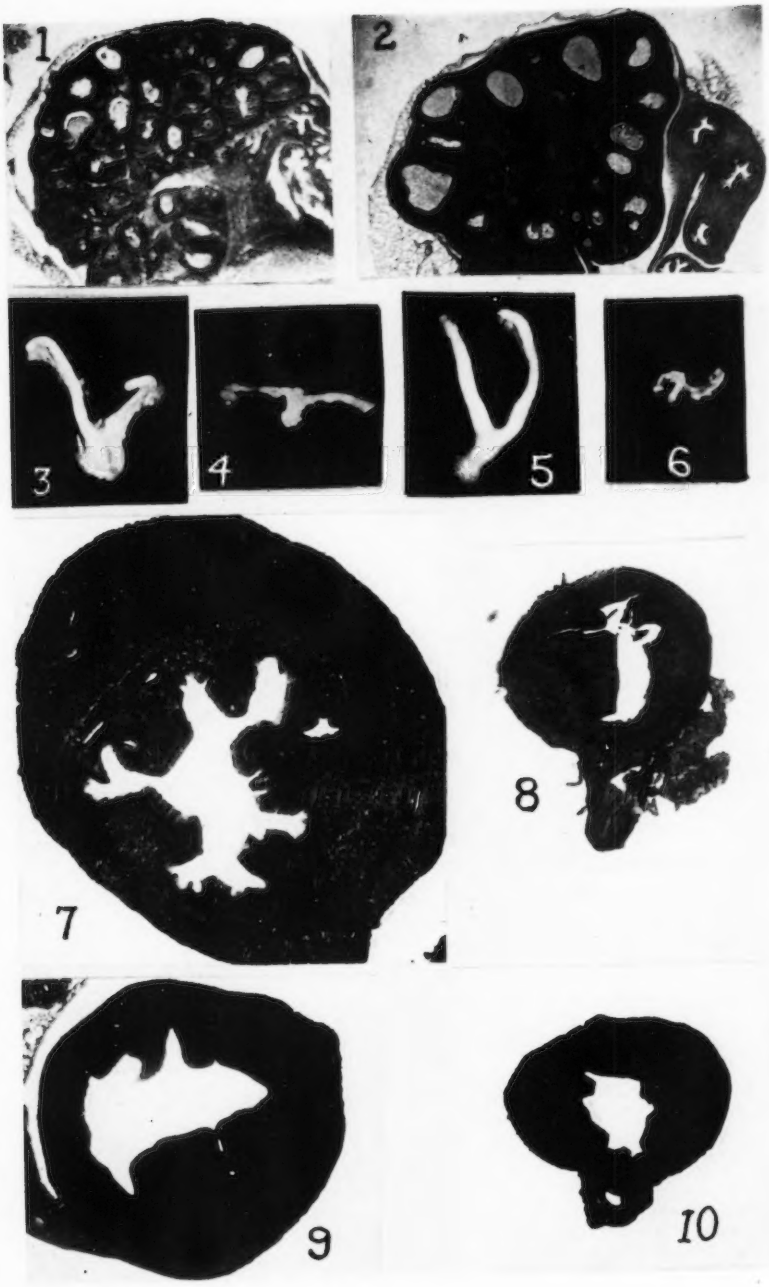
Fig. 6. Uterus of its litter-mate control (about × 2).

Fig. 7. Cross-section of uterus shown in figure 3. × 50.

Fig. 8. Cross-section of uterus shown in figure 4. × 50.

Fig. 9. Cross-section of uterus shown in figure 5. × 50.

Fig. 10. Cross-section of uterus shown in figure 6. × 50.



Figs. 1 to 10

more potent extracts were available, or if the injections were begun at an earlier age period. Our cases were negative for the sexual organs at the 18, 20 and 25-day ages, however, even when large doses of extract were administered over periods up to two weeks. Increase in pituitary size did appear, nevertheless, at 20 days.

From the 28-day-old period onward there were rapidly increasing signs of precocious sexual activity in the female. The data derived from 14 litters, comprising 63 injected animals plus their litter-mate controls, may be observed at a glance in table 1.

There was no excessive luteinization noted in the ovary such as that observed by Claus (1931), and by Fevold, Hisaw and Leonard (1931). The normal maturation process, and the normal formation of lutein material following the rupture of the follicle, apparently took place in all cases in which cortico-adrenal extract was administered.

In three rats which were injected with 1 cc. of extract daily for 2 weeks, and were 28 days old when sacrificed, the uterine hypertrophy was most pronounced. Careful measurements were made in two of these cases with the following results (see also figs. 3, 4, 5 and 6):

Controls:	C-1	Uterine length 7 mm., diameter 0.660 mm.
	C-2	Uterine length 10 mm., diameter 0.527 mm.
Experimentals:	E-1	Uterine length 19 mm., diameter 2.178 mm.
	E-2	Uterine length 19 mm., diameter 1.656 mm.

The sloughing of cornified cells from the endometrium was indicative that ovulation had occurred. The endometrial tissues showed marked thickening, and there was every evidence of maturation. None of these characteristic changes were seen in the uteri of the controls; the cell layers of the latter were by contrast much smaller and quite immature.

The testis. Photographs are shown of sections through the central portion of the testis (figs. 15 and 16). There was usually no apparent increase in the size of the organ in the injected animals. In two cases there was, however, marked enlargement; this small percentage of the total cases scarcely warrants the conclusion, however, that general hypertrophy of the testis follows cortico-adrenal extract injection.

Fig. 11. Longitudinal section through the hypophysis of a 20-day-old rat which received 1 cc. of extract per 10 grams of rat daily for 5 days.

Fig. 12. Longitudinal section through the hypophysis of its litter-mate control (same magnification).

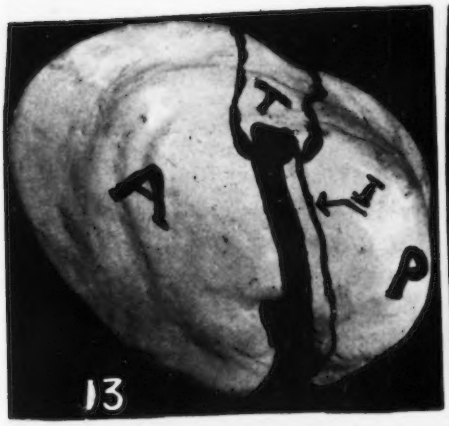
Fig. 13. Reconstruction of gland shown in figure 11.

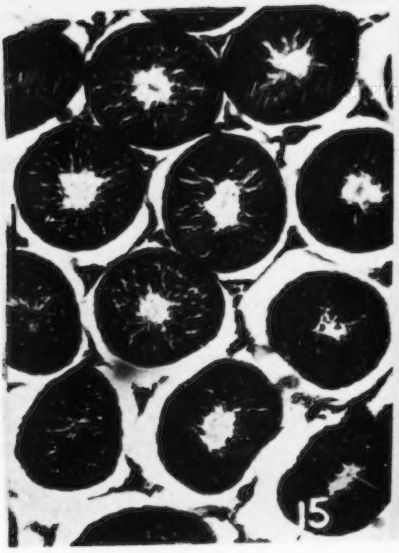
A. Pars anterior. P. Pars posterior. I. Pars intermedia. T. Pars tuberalis.

Fig. 14. Reconstruction of gland shown in figure 12.

Fig. 15. Section through the testis of a 35-day-old control animal.

Fig. 16. Section through the testis of its litter-mate which received 1 cc. of extract daily for 2 weeks (same magnification).





Figs. 11 to 16

In histological section, the tubules appear larger in diameter, and the tissue presents a closely-packed or compressed appearance. This may probably be attributed to increased tubular diameter and length. This appearance, it may be remarked, is typical of the testes of rats injected with macerated pituitary gland (Corey, 1930).

Spermatogenesis is present from 35 days of age onward. In the testes of 42-day-old rats there may be found indications of spermatogenesis in some of the tubules of control animals. By count, determinations were made in these rats of the percentage of tubules exhibiting spermatogenesis in the experimental and control (litter-mate) animals, with the following result:

42-day control animals, spermatogenesis in 16 per cent of the tubules
42-day experiments, spermatogenesis in 30 per cent of the tubules

Spermatogenesis was first seen in excess of that noted in controls at 35 days of age, following the daily injection of 1 cc. of the extract throughout two weeks. It is thus apparent that the testis does not respond to the extract to the same extent as does the ovary. The diameter of the tubules of the epididymis was somewhat increased, it may be noted, in the injected cases.

Pituitary gland. The photographs of models and sections through the median portion of the pituitary (figs. 11, 12, 13 and 14) well illustrate the effects which were produced. There was hypertrophy throughout the gland in the injected animals. Reconstructions were made to render the observations on this point more apparent. The anterior lobe was most affected; an increase in the eosinophilic cells, particularly, caused the marked increase in size.

The gland was found to be abnormal in shape, possibly due to pressure differences—a condition which was brought out very clearly in the reconstructions. The anterior lobe presented a wedge-shaped appearance. The pars intermedia had become flattened antero-posteriorly, so that it took the form of a ribbon-like web of cells rather than the normal tissue mass. The pars tuberalis was apparently unaffected; it retained its normal position and volume. Apart from the hypertrophy, the tissues of the experimental gland appeared normal, and the variations may be considered proportional rather than structural.

The effect on the pituitary first appeared in 20-day-old animals, which had been injected from the 15th day onward. The dosage given was 1 cc. daily per 10 grams of body weight of rat.

Thyroid gland. No significant changes were apparent in the thyroid of the injected animals, regardless of the dosage or the length of the experimental period. In both experimental and control series the glands showed numerous small follicles, which were normally distended with colloid.

The parathyroids of the injected animals likewise appeared to be similar to those of the controls.

Adrenal glands. In these glands, also, no marked changes were evident in the extract-treated rats. Some hypertrophy of the cortex appeared to be present, but evidence of this was so slight that no reconstructions were made. Certainly no degeneration of the gland took place. We were unable to observe any hypertrophy effects such as those reported by Papanicolaou (1931) to follow the injection of the urine of pregnant women and of cattle, in guinea pigs.

Weights of animals. Although the changes were small, the weights of the injected animals were noticeably increased following the injection of extract of the adrenal cortex. The increases are significant when considered in relation to the small size of the animals used, the brief periods of injection, and the constancy of their occurrence. Observations of weight changes were made in litters as follows:

Litter 2, injected 5 days, 1 cc. per 10 grams of rat; killed at 20 days of age. Controls, 55, 45, 45, 45 grams; average 47 grams. Experimentals, 50, 55, 60, 50, 45 grams; average, 52 grams.

Litter 7, injected 14 days, 1 cc. extract daily; killed at 28 days of age. Controls: 30, 25, 25, 25, 25 grams; average 26 grams. Experimentals, 35, 30, 30, 35, 30 grams; average 32 grams.

Litter 11, injected 14 days, 1 cc. extract every second day; killed at 34 days of age. Controls, 45, 50, 45, 45 grams; average 46 grams. Experimentals, 55, 50, 56, 50, 50, 55 grams; average 51 grams.

Litter 13, injected 14 days, 1 cc. extract every second day; killed at 37 days of age. Controls, 45, 50, 45, 45 grams; average 46 grams. Experimentals, 50, 50, 55, 50, 45 grams; average 51 grams.

It is apparent therefore that in every litter examined the injected animals averaged heavier than the controls. The average weight of the 17 control animals of this series was 43 grams, and of the 22 experimental animals 49 grams—a notable increment of 14 per cent in favor of the extract-injected cases over their litter fellows.

Discussion. The report of Hoskins and Hoskins (1916) that in 26 animals they obtained testicular hypertrophy following the feeding of desiccated adrenal gland may be recalled. They state that the ovaries were "larger in a few cases." Our observations with cortico-adrenal extract are not in accord with those of the above workers, since the ovaries invariably showed more striking changes than did the testes. McKinley and Fisher (1926) likewise reported testicular enlargement following the feeding of cortical material. Singularly, perhaps, a great deal of clinical evidence points to augmentation of the male elements in cases of hyper-

trophy (tumors) of the adrenal cortex. If the tumor growth occurs in the female, indeed, there frequently is observed a tendency towards reversion of type.

It seems likely that the extracts we have used contain more than one hormone—probably at least two hormones. One of these may be concerned with the maintenance of adrenalectomized animals in the normal condition. Normal control of the sex organs may be maintained in part by a second hormone. Rather than assume a further division of duties between the two hormones for the regulation of male and female activities (and thus postulate that three hormones are contained in the adrenal cortex), it would appear reasonable to consider that the effects of the cortical sex-stimulating factor represent degrees and not qualities of stimulation. Perhaps an "adrenal sex-hormone" first causes increased activity of the gonads leading to femaleness in the organism, and then continued or cumulative excitation results in a dominating appearance of masculine characteristics.

Maleness in the organism having once come into predominance might not be so readily further excited. The consideration that sex is "a matter of quantity," and also the occurrence of "free-martins" in animals, would appear to support this hypothesis. That there are many apparent difficulties about this conception is appreciated, but they do not warrant discussion at this early time. It may be the case, nevertheless, that retention of a specific female-activating hormone is particularly favored in the long-drawn-out method of extraction of the adrenal cortex which we employed.

The similarity of our results to those obtained by the transplantation of hypophyseal tissue from the anterior lobe may be mentioned. A comparison of our plates with those of Smith (1927), and Smith and Engle (1927), is very striking. Effects produced by the injection of fresh macerated pituitary gland into young rats (Corey, 1930) are also similar to those obtained in the present investigation.

Claus (1931), and also Fevold, Hisaw and Leonard (1931), have recently reported the presence of a luteinizing hormone in the anterior pituitary lobe. These workers state that this hormone cannot act upon the immature ovary, but that the latter must first be stimulated (activated?) by a second and distinct gonad-stimulating hormone.

Our results appear to indicate that the pituitary gland is initially stimulated to increased activity by the injection of cortico-adrenal extract. Possibly an enhanced pituitary secretion then acts on the gonads. This viewpoint may perhaps arise particularly because of the recent well-approved observations on pituitary activity. It is nevertheless wholly possible that hypophyseal enlargement following cortical extract injection is only incidental, and not specifically related to the gonadal changes reported herein.

SUMMARY

Precocious maturation of the sex glands of young albino rats has been produced by the injection of cortico-adrenal extract (Swingle-Pfiffner) prepared in this laboratory. Marked effects were observed in the ovaries of 28-day-old animals. The appearances of early maturity in the testes were not so striking, and occurred later.

Hypertrophy of the pituitary gland, especially of the anterior lobe, appeared in 20-day-old rats. The possibility that the effects on the gonads are secondary to enhanced pituitary secretion is entertained.

No significant changes were found in the thyroid or the adrenal glands, either in size or structural relationships.

An increase in weight in excess of that of control rats was observed in animals injected with the extract.

The probability that at least two hormones may be derived from the cortico-adrenal tissues is further discussed.

BIBLIOGRAPHY

Britton, S. W. and H. Silvette. 1931a. Science, lxxiii, 322, 373.
1931b. This Journal.
Bullock, W. and J. Sequiera. 1905. Trans. Path. Soc. London, lvi, 189.
Claus, P. E. 1931. Physiol. Zool., iv, 36.
Corey, E. L. 1930. Physiol. Zool., iii, 379.
Corey, E. L. and S. W. Britton. 1931. Science, lxxiv, 101.
Fevold, H. L., F. L. Hisaw and S. L. Leonard. 1931. This Journal, xcvii, 291.
Glynn, F. 1912. Quart. Journ. Med., v, 157.
1921. Journ. Obstet. and Gyn., xxviii, 23.
Guthrie, L. and W. d'E. Emery. 1907. Trans. Clin. Soc. London, xl, 175.
Hartman, F. A. and F. A. Brownell. 1930. Proc. Soc. Exp. Biol. and Med., xxvii, 938.
Hartman, F. A. and G. W. Thorn. 1930. Ibid., 94.
Hartman, F. A., K. A. Brownell and W. E. Hartman. 1930. This Journal, xcv, 670.
Hoskins, R. A. and A. D. Hoskins. 1916. Arch. Int. Med., xvii, 584.
Jaffe, H. L. and D. Marine. 1923. Journ. Exp. Med., xxxviii, 93, 107.
McKinley, E. B. and N. F. Fisher. 1926. This Journal, lxxvi, 268.
Müller, C. 1931. Endokrinol., viii, 5.
Nagle, W. 1836. Müller's Arch., p. 365.
Papanicolaou, G. N. 1931. Anat. Rec., xlviii, 59.
1931. Proc. Soc. Exp. Biol. and Med., xxviii, 807.
Schiffer, A. L. and L. B. Nice. 1930. This Journal, xcv, 292.
Smith, P. E. 1927. This Journal, lxxx, 114.
Smith, P. E. and E. T. Engle. 1927. Amer. Journ. Anat., xl, 159.
Swingle, W. W. and J. J. Pfiffner. 1931a. This Journal, xcvi, 153, 164, 180.
1931b. Proc. Soc. Exp. Biol. and Med., xxviii, 510.
Zwemer, R. L., F. J. Agate, Jr. and H. A. Schroeder. 1931a. Proc. Soc. Exp. Biol. and Med., xxviii, 721.
Zwemer, R. L. and R. C. Sullivan. 1931b. Ibid., 723.

THE ORAL ADMINISTRATION OF CORTICO-ADRENAL EXTRACT[1]

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The effectiveness of cortico-adrenal extract when given by subcutaneous, intraperitoneal or intravascular injection has been well demonstrated (Swingle and Pfiffner, 1931; Britton and Silvette, 1931a, b). We have considered recently some effects produced by the extract when given by mouth. As opportunity allowed, a few animals were given extract by stomach tube during our earlier investigations, when certain batches of the material had proved of considerable potency by the intraperitoneal test. Although the very limited availability of glandular supplies, and hence of the finished extract, has somewhat restricted our observations on the oral treatment of adrenal insufficiency, the results have been characteristically positive.[3]

The extract was given in the same concentration as used in other experiments in this series, i.e., 100 cc. were prepared from each 4 kilograms of fresh whole adrenal glands. At first, attempts were made to introduce the extract by stomach tube. It was appreciated that adrenalectomized animals are very readily upsét and often vomit spontaneously, and great care was therefore taken in using this form of treatment. As small an

[1] Reported at a meeting of the joint session of the Federation of American Societies for Experimental Biology at Montreal, April 11, 1931.

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[3] Although it invariably yields potent extracts, the method of Swingle and Pfiffner is very costly in both time and material. About two weeks are taken in the preparation of one batch of extract (100 cc.). The actual expense of this amount is about $30.00. We have given up to 100 cc. to one cat in 24 hours; frequently we have used 30–40 cc. on an animal in one day, in attempting to secure positive results. Swingle and Pfiffner (1931) have likewise found it necessary to use large amounts of material. To produce effects on man we have employed very large amounts of extract, as also have others (Rowntree, Greene, Swingle and Pfiffner, 1931). There is great need of a quicker and less costly method of extraction; one, too, which will produce a much greater percentage yield.

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amount as 10 cc. was often vomited, however, and the method had to be abandoned as uneconomical. All attempts to give the material in milk or with salmon juice were unsuccessful; adrenalectomized animals with symptoms, indeed, do not fancy these dishes even *sans* medicament. The method of spoon-feeding was fairly satisfactory, but again wasteful because of frequent failure to swallow the extract, and loss from the sides of the mouth. Introduction of the extract by means of a syringe, with a short piece of heavy-walled rubber tubing attached, we have found to be most successful.

Influence of extract administered by mouth on survival after adrenalectomy. That it is possible to keep adrenalectomized animals alive for abnormally long periods when cortico-adrenal extract is given by mouth is apparent from cases 388 and 444. Protocols on these and a few of the other animals studied are given later in brief.

The general observations on cat 388 are noteworthy: the animal showed the usual muscular symptoms of adrenal insufficiency six days after operation; it refused food, it had lost weight, and also suffered from an eye infection which suggested the onset of "snuffles," a condition often fatal in cats. Treatment orally with cortico-adrenal extract restored the animal to the normally active condition; it began to take food again, gained nearly 10 per cent in weight (became heavier, indeed, than when observed at operation), and in a few days the eye infection disappeared. The blood-chemistry changes, which are considered separately later, consolidated the proof that extract of the adrenal cortex is efficacious when given by the oral route. The animal remained in apparently good condition while the extract was kept up; it lost weight and eventually died showing typical symptoms of insufficiency some days after extract administration was withdrawn. The period of survival after operation, although only 18 days, was considerably longer than that observed in untreated controls (Britton and Silvette, 1931b).

The large amounts of extract apparently necessary to be given by mouth to keep adrenalectomized cats alive appeared to us prohibitive. Rats are unsuitable in surviving experiments for many reasons (Britton, 1930). Recently we have used kittens, from one to two months old, in our operations. These animals we found to be remarkably responsive to treatment.

Kitten 444 furnishes an interesting story of recovery by extract after many setbacks. Twice it was restored from convulsions by cortico-adrenal extract. It was often stimulated to take food within an hour or so after symptoms had appeared, when extract had been given. After a weight loss of 50 grams (470 to 420 grams), it regained 60 grams under extract treatment. Large amounts of the extract were necessary, considering the small size of the animal, to keep it well. It was kept alive for 15 days after

convulsions first appeared. Symptoms redeveloped rapidly and death occurred 24 hours after the last dose of available extract had been given.

Six other *normal* kittens, we have discovered since carrying out the above experiment, survived under our laboratory conditions during the the hot summer months for periods between 5 and 15 days. Untreated adrenalectomized kittens have lasted in a few cases tested between 3 and 6 days only.

Restoration after the onset of definite insufficiency symptoms. In seven animals in which definite symptoms of adrenal insufficiency had been allowed to develop, complete recovery to the normally active condition was observed on the administration of cortico-adrenal extract by mouth. Another animal, cat 409, showed recovery from weakness, but there were later indications that accessory cortical tissue was present in this case, and it is not considered positive. These eight cases represent all the animals which received treatment in these experiments; the administration of cortico-adrenal extract by mouth was effective in bringing about recovery in every one of the series studied.

Symptoms were allowed to set in a number of times in some cases, and in these oral treatment was invariably efficacious. Cats 388 and 423 were each restored on two occasions, cat 428 three times, and cat 444 four times.

Usually the animals did not show an interest in food for two to several hours after extract had been given; cat 444 greedily took raw liver on two occasions, however, within an hour after suffering from a severely prostrated convulsive condition. Perhaps no more positive sign of restoration of an adrenalectomized animal in the terminal stages of insufficiency could be desired than the stimulation to take food, such as we observed several times after extract was given by mouth.

Convulsions are invariably indicative of the terminal condition in adrenalectomized animals. In by far the greater number of a series of over 100 cases, we have observed that death ensues within one or two hours after convulsive seizures appear. Frequently two or three fits will result in death in half an hour. On several occasions after cortico-adrenal extract has been given by mouth, convulsions have been abolished, and in an hour or so, sometimes in less time, the animals have appeared normal. Cat 406 sat up and seemed well an hour after 10 cc. extract were given. Cat 413 was much improved by the extract within an hour after several convulsive fits had been observed; recovery from convulsions which reappeared later was definitely apparent in two-and-a-half hours. Cat 444 sank into convulsions twice, and was readily restored; once it ate ravenously within an hour after the extract had been given.

Changes in blood sugar, non-protein nitrogen and cell volume. In several animals showing symptoms of adrenal insufficiency marked changes in

the above important blood constituents, towards or to the normal levels, were observed after cortico-adrenal extract had been given by mouth.

Animal 386, whose blood sugar had fallen after adrenalectomy to 51 mgm. per 100 cc., showed augmentation of the sugar level, 18 hours after the oral administration of extract, to 80 mgm. per cent. The blood non-protein nitrogen level was decreased from the high figure of 142 mgm. per 100 cc. on March 19 to 44 mgm. the next day. With these blood changes there was noted a remarkable improvement in the animal's condition. Discontinuance of the extract quickly resulted in diminution of the sugar and augmentation of the non-protein nitrogen levels, and death occurred two days later.

Frequent observations were made on cat 388; it obviously took large amounts of extract to keep the blood constituents of this animal within the normal levels. Even the smaller amounts of extract used tended to induce changes, however, towards the normal. In the afternoon of April 2, when 95 cc. of cortico-adrenal extract were given by mouth in an attempt to shift the percentage of the blood constituents, the following changes were observed: The blood sugar increased from 56 to 92 mgm. per 100 cc., the non-protein nitrogen fell from 50 to 24 mgm. per 100 cc., and the cell-volume diminished from 40 to 28 per cent.

The changes after adrenal removal in cat 413 were as follows: The blood sugar fell from 70 to 49 mgm., the non-protein nitrogen rose from 32 to 95 mgm., and the cell-volume from 34 to 46 per cent. Such changes have been shown to go hand-in-hand with the later stages of adrenal insufficiency. Extract administration by mouth was found to increase the blood sugar from 49 to 78 mgm.; it reduced the non-protein nitrogen from 95 to 48 mgm., and it reduced the cell-volume from 46 to 32 per cent.

The considerations noted above are illustrated in the following protocols:

Cat 386, female; weight 1.82 k. Fasting blood non-protein nitrogen, 24 mgm. per 100 cc. March 17, 1931, bilateral adrenalectomy.

March 18, blood sugar 58 mgm., non-protein nitrogen 71 mgm.

March 19, 11 a.m., blood sugar 51 mgm., non-protein nitrogen 142 mgm.; cat showing slight weakness. At 4 p.m., animal just able to walk; will not eat; 25 cc. cortico-adrenal extract no. 42 given by stomach tube at 4:10 p.m. Cat appeared stronger, walking about well, at 5 p.m.

March 20, 10 a.m., condition good; blood sugar 80 mgm. At 10:30, gave 25 cc. extract 42 by stomach tube, but cat vomited considerable amount immediately after; 20 cc. extract given by spoon-feeding; no vomiting followed. At 4:20 p.m., blood sugar 87 mgm., non-protein nitrogen 44 mgm.

March 21, 10:00 a.m., walks about normally; blood sugar 56 mgm., non-protein nitrogen 105 mgm. Extract discontinued.

March 22, 9 a.m., condition good. 7 p.m., appeared well.

March 23, 8:45 a.m., found dead in cage; weight 1.56 k. Autopsy showed slight pancreatic congestion, and three ulcerated areas, two of which had healed, in gastric mucosa. No accessory cortical tissue was present.

Cat 388, male; weight 1.81 k. Adrenalectomized on March 23, 1931.

Weakness in hind limbs, food refused, on March 29; eyes infected; weight 1.80 k. Blood sugar 52 mgm. non-protein nitrogen 45 mgm., cell volume 49 per cent; 20 cc. cortico-adrenal extract 47 spoon-fed at 3:45 p.m. Cat much improved, appeared normally active, at 7:45 p.m. At 8 p.m., blood sugar 66 mgm., non-protein nitrogen 53 mgm., cell volume 45 per cent. Cat ate meal of salmon just after blood samples taken.

March 30, 9 a.m., in vigorous condition. 10 a.m., blood sugar 66 mgm., non-protein nitrogen 48 mgm., cell volume 41 per cent.

March 31, 9 a.m., cat active, well; weight 1.82 k. 3 p.m., blood sugar 60 mgm., non-protein nitrogen 30 mgm., cell volume 42 per cent. At 3:30, 22 cc. extract by mouth.

April 1, 9 a.m., animal very active; eye condition cleared.

April 2, appears in fair condition. At 11:45 a.m., blood sugar 56 mgm., cell volume 40 per cent. Gave large quantity of extract by mouth (spoon-fed) in attempt to change the percentage of blood constituents: 30 cc. no. 44 at 2:30 p.m., 25 cc. at 3:30, 25 cc. at 4:30. At 5:30, blood sugar 57 mgm., non-protein nitrogen 50 mgm., cell volume 29 per cent. At 6:00 p.m., 15 cc. extract, orally. At 6:45, blood sugar 92 mgm., non-protein nitrogen 24 mgm., cell volume 28 per cent.

April 3, cat well. 11 a.m., blood sugar 73 mgm., non-protein nitrogen 34 mgm., cell volume 25 per cent.

April 4, condition excellent, eating well; weight 1.96 k. No further extract given.

April 5–7, cat appeared normal. April 8, weight 1.8 k. 11 a.m., blood sugar 57 mgm., non-protein nitrogen 53 mgm., cell volume 36 per cent.

April 10, 9 a.m., cat appeared normal. At 7:30 p.m., found in extremis; died with typical insufficiency symptoms at 7:40 p.m. Autopsy findings negative. Weight at death 1.79 k.

Cat 413, female, weight, 1.7 k. April 20, 1931, fasting blood sugar 70 mgm., non-protein nitrogen 32 mgm. per 100 cc., blood cell volume 34 per cent. Both adrenals removed at 11 a.m.

Next morning, herniation from abdominal wound was observed; a repair operation was performed under ether.

April 22, 9 a.m., cat appeared well. At 2:20 p.m., found prostated, occasional convulsions. Blood sugar 49 mgm., non-protein nitrogen 95 mgm., cell volume 46 per cent. Gave 30 cc. extract 52 at 2:30 p.m. Several convulsive fits followed, also attempts to vomit. At 3:30 p.m., animal appeared much improved, alert. At 6:30 p.m., convulsions recurred; attempted to give extract by mouth, but several cubic centimeters were regurgitated. Notable improvement in condition at 9 p.m.; 30 cc. extract 53 by mouth. At 9:45 p.m., blood non-protein nitrogen 48 mgm., cell volume 42 per cent.

April 23, 9 a.m., cat well, walking about cage; blood sugar 78 mgm., non-protein nitrogen 40 mgm., cell volume 37 per cent. Ate salmon after blood samples taken. Twenty cubic centimeters extract 51 given at 9 p.m. Cat very active, took milk, at 10:30 p.m.

April 24, 8:30 a.m., found dead. Post mortem showed extensive abdominal infection.

Cat 444, male, 0.47 kilo. Fasting blood sugar 85 mgm. per 100 cc. Adrenalectomized on May 15, 1931.

May 18, 8:30 a.m., found prostrated. At 9:05, blood sugar 46 mgm. per 100 cc. Weight, 0.45 k.; gave 10 cc. extract 64 by mouth. 10:20 convulsions. Gave further

10 cc. extract 63 at 10:25. At 11:00 cat much improved, started to walk about; appeared as if it would eat salmon, but was not allowed to do so. At 12:30, blood sugar 48 mgm. At 2:45 p.m., blood sugar, 101 mgm.; cat wanted to eat salmon presented. Very active at 5 p.m.; blood sugar 119 mgm. 7:30 p.m., blood sugar 159 mgm.; allowed animal to eat salmon.

May 19, 9 a.m., cat active; blood sugar 86 mgm. Gave 10 cc. extract 63 at 12:30 p.m.

May 20, 8:30 a.m., condition unchanged; blood sugar, 105 mgm. Gave 10 cc. extract 63 at 10:15 a.m. At 4 p.m., blood sugar 90 mgm. At 11:30 p.m., 10 cc. extract 65.

May 21, 8:30 a.m., condition good; gave 5 cc. extract 65. 9:30 p.m., 6 cc. extract 65.

May 22, condition good throughout day. Gave 6 cc. extract at 10:30 p.m.

May 23, condition unchanged; gave 5 cc. extract at noon.

May 24, 9 a.m., slight weakness, food refused; weight 0.42 k. At 9:50 gave 4 cc. extract 66 by mouth. At 10:20, cat strikingly improved, very active; ate salmon and drank milk. At 10 p.m., 5 cc. extract 66.

May 25, 9:15 a.m., found in convulsions; blood sugar 43 mgm.; weight 0.43 k. Gave 10 cc. extract 66 at 9:25. At 10:10, cat ate 30 grams chopped liver. At 5 p.m., blood sugar 147 mgm. Ate 25 grams liver. Weight after eating 0.48 k.

May 26, condition excellent. Gave 6 cc. extract 66 at 11 a.m. Cat took 40 grams liver at 11:10. At 4:25 p.m., gave 5 cc. extract 66. Weight 0.48 k.

May 27, in good condition; gave 10 cc. extract 66 during day.

May 28, appeared well; weight 0.44 k. Gave 20 cc. extract by mouth during day.

May 29, condition unchanged; gave 12 cc. extract 67.

May 30, very active; gave 10 cc. extract 67.

May 31, 10 cc. extract 67; same condition.

June 1, 8:30 a.m., slight weakness; animal refused food. Gave 15 cc. extract 67 during the morning. In the early afternoon, cat ate 20 grams of liver; very active at 5 p.m. Weakness shown in hind limbs at 11 p.m. No extract available.

June 2, 8:30 a.m., cat very weak; convulsions developed rapidly; no extract. At 9, death with typical symptoms of insufficiency. Weight at death, 0.42 k. Autopsy revealed no cortical accessory tissue.

That the hormone of the adrenal cortex which is responsible for maintaining the lives of adrenalectomized animals is not destroyed wholly, if at all, by the gastro-intestinal mucous membranes, is clear from the foregoing experiments. Apparently there is agreement in this respect with the known slight action of adrenalin by mouth. Considerably larger amounts of cortico-adrenal extract were necessary when the material was given by mouth to produce effects comparable to those observed when the material was injected intraperitoneally. Roughly, it appeared that from three to five times the dosage was required.

In view of the undoubted influence of the extract, and the widespread effects which it is already known to produce, it seems likely that its use in the medical field will soon find wide application. With improved methods of preparation and the achievement of a more potent product, it is possible that oral treatment may be of distinct service. In cases in which prolonged

treatment is demanded, at least, the oral route may be of preferential utility.

The positive results reported herein suggest that inquiry be made into the possible effects of feeding adrenal gland in cases of adrenal insufficiency, as well as in normal animals. Hitherto unsuccessful attempts with this form of treatment are possibly explained by the lack of adequately fresh glandular material, or perhaps by the complicating presence of the medulla of the gland.

We have tried the feeding of adrenal gland on a few operated animals without present success, perhaps because of the vomiting which followed. This we have attributed to the adrenalin content of the tissues. Even when the medulla is well scraped out before extraction, it may be observed, considerable amounts of adrenalin are carried over with cortico-adrenal extract, through the long-drawn-out process, until the permutit or other methods of eliminating it are employed. In accord with many observations, it seems that adrenalin may thus permeate through into the cortical tissues shortly after death of the animal. Hope is nevertheless entertained that improved methods of feeding adrenal cortex may yet be found to influence normal and also abnormal bodily activities.

Adrenalin and glucose administration. These substances have been shown to produce slight results when given intraperitoneally to adrenalectomized animals (Britton and Silvette, 1931b); the changes in the cases studied were nevertheless temporary, and at the most lasted only a day or so. The slight therapeutic effect of adrenalin by mouth on normal animals, and the ready absorption of glucose from the gastro-intestinal tract, suggested that these materials might be of some value.

In control experiments adrenalin solution in concentration and amount similar to that in cortico-adrenal extract, and also glucose solution, were tested. The results were in all cases, however, quite negative. It was clear that both adrenalin and glucose by mouth had no ameliorative effect on even the earlier symptoms of adrenal insufficiency. In all cases, indeed, the animals died within a day after treatment was started. The large amounts of fluid represented were not of significant influence. The solutions of adrenalin used did not affect the blood sugar level; the change after glucose administration indicated, however, that this material had been absorbed.

SUMMARY

The effectiveness of cortico-adrenal extract by mouth is demonstrated. It prolongs considerably and possibly indefinitely the lives of adrenalectomized cats, and brings about complete recovery from even the severest symptoms of adrenal insufficiency, when given by the oral route. The low blood-sugar values found in adrenalectomized animals are markedly

increased, and the high non-protein nitrogen values and blood cell volumes are reduced towards or to the normal levels, during the recovery period. Animals that have refused food may be induced to eat within an hour after extract administration. These observations are in keeping with those produced by intraperitoneal injection of the material.

Large amounts of the extract were necessary by mouth, approximately three to five times the intraperitoneal dosage, in order to produce comparable results.

Adrenalin given by mouth in similar concentration to that found in cortico-adrenal extract, and also glucose solutions, have no noteworthy effect on adrenal insufficiency.

BIBLIOGRAPHY

BRITTON, S. W. 1930. Physiol. Rev., x, 617.

BRITTON, S. W. AND H. SILVETTE. 1931a. Science, lxxiii, 322, 373.
1931b. This Journal, xcvii, 507.

ROWNTREE, L. G., C. H. GREENE, W. W. SWINGLE AND J. J. PFIFFNER. 1931. Journ. Amer. Med. Assoc., xcvi, 231.

SWINGLE, W. W. AND J. J. PFIFFNER. 1931. This Journal, xcvi, 153.

STUDIES IN THE PHYSIOLOGY OF EXERCISE

VII. The Modification of Colonic Motility Induced by Exercise and Some Indications for a Nervous Mechanism[1]

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In *A Treatise of the Science of Muscular Action* (1794) John Puch, a London anatomist, quotes unnamed earlier authorities as follows: "Exercise helps to throw down wind from the bowels and attenuates the contents of the stomach." "Exercise also serves at once as an evacuant, and a diversion by which artifices the humours are put into condition of flying off without the danger of bringing on spasms." Though not always as graphically, each modern day system of the practice of medicine lists as one of the etiological factors in constipation "the sedentary life" or "lack of exercise." Some writers see in a strengthened abdominal musculature the facilitation of defecation while others attribute the beneficial effects of exercise to the massaging action of the exercise movements.

The chronic effect of a very active physical life program on the gastrointestinal activity level though no doubt the problem of greatest interest, has to our knowledge not received careful consideration by any investigator. Concerning the immediate effects of a bout of exercise on gastric motility numerous workers have reported contradictory results. As far as we know, however, the colon has not received such attention.

In 1926 we began in this laboratory, a series of studies which we hoped would throw light on the immediate effects of a short period of strenuous exercise on the motility of various parts of the digestive tube. We explored first the jejunum and then the stomach and colon. Because the colon response was the most constant and characteristic we decided to pursue it to the limit before tackling the more fickle stomach and small intestine. We found the balloon method entirely unsuited for jejunal studies. In this paper we present in detail our findings on the effect of exercise on the motility picture of the colon together with certain conclusions concerning the responsible nervous mechanisms. A portion of our findings has already been reported in preliminary fashion (Steinhaus and DeYoung, 1931).

[1] For the period beginning September 1, 1929 a portion of the cost of this study was borne by a grant from The Carnegie Corporation of New York.

TABLE 1

DOG	DATE AND NATURE OF OPERATION	NUMBER OF TRACINGS	NUMBER OF EXERCISE PERIODS	PERCENTAGE OF EXERCISE PERIODS SHOWING RISE IN ACTIVITY		PERCENTAGE OF EXERCISE PERIODS FOLLOWED BY PERIOD OF REDUCED ACTIVITY	
				In colon	In pouch	In colon	In pouch
14	5-15-28 Cecostomy	36	69	42		48	
	2- 8-29 Denervation (First method)	13	38	55		18	
15	3-6-28 Cecostomy	27	45	80		64	
	7-12-28 Denervation (First method)	5	6	100		66	
16	10-28-28 Cecostomy Denervation (Second method)	22	47	15		22	
17	11-22-28 Cecostomy Denervation (Second method)	9	37	19		19	
18	6-25-29 Cecostomy	11	32	78		66	
	12-27-29 Denervation (Third method)	19	45	69		66	
28	9-14-30 Cecostomy and Thiry colonic pouch	5	24	54	0	62	67
	9-29-30 Pouch denervated	6	21	71	0	76	85
29	10-29-30 Cecostomy and Thiry colonic pouch	7	31	29	0	74	64
	4-20-31 Section colon "Low cut" to make "double cut" dog	3	15	0		48	
30	4-21-31 Cecostomy Section colon "high cut"	2	12	50		33	
	5-2-31 Section colon "Low cut" to make "double cut" dog	2	9	0		33	
31	5-18-31 Cecostomy Section colon "low cut"	4	20	40		45	

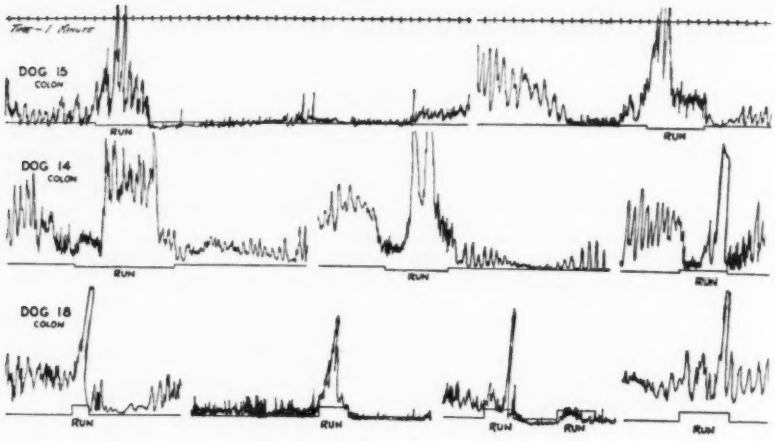

Fig. 1. A number of typical records of colon motility in three normal dogs showing the characteristic "exercise rise" and the "after effect." The record of dog 18 shows in one instance the failure of appearance of the rise after repeated exercise periods.

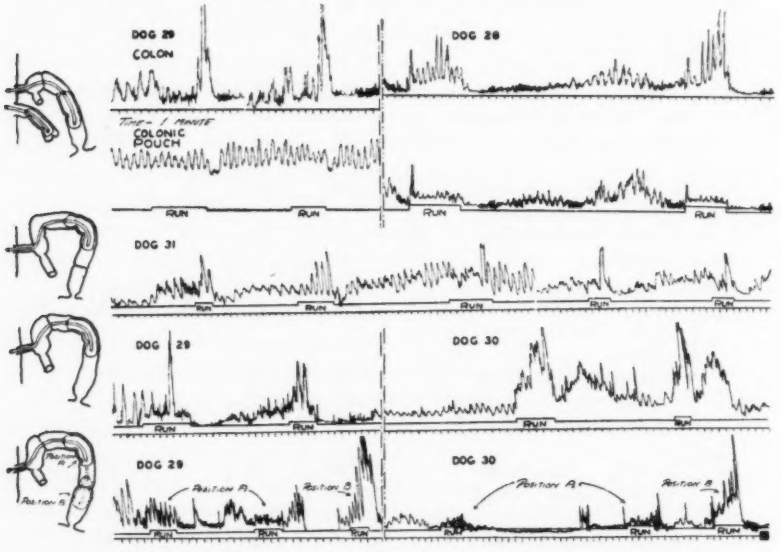

Fig. 2. Records of the effect of running on motility of the colon after it had been transected at various levels. Diagrams at the left indicate the nature of the surgical preparation in each instance and the location of the balloon. The levels of transection are indicated by the transverse lines. The sharp rise regularly shown at the beginning of a running period in the tracings of dog 28 is due to contraction of abdominal musculature associated with barking (a habit of this dog at the beginning of each exercise period).

EXPERIMENTAL METHODS. Dogs, completely recovered from operations to produce suitable fistulae and other surgical interventions, were studied by the balloon method as modified for use in intestinal work by Ivy and Vloedman (1923). A special water manometer designed by Mr. Walter T. Anderson, a student in this laboratory and described by Steinhaus et al., (1928) was used to record the motility on a long paper kymograph. The manometer is built on the principle of the hydraulic press so that the recorded rises of the marker are approximately one-sixth as high as the actual pressure changes.

For colonic studies a cecostomy was produced. In a specific experiment the dog stood on the treadmill beside the kymograph while records of an hour or more of normal motility were secured. Then without any other change the motor driven treadmill was started and the dog caused to run uphill (about 22 per cent grade) at the rate of a mile in twelve minutes for a period usually six minutes in length. The treadmill was then stopped and a suitable post-exercise record secured. (Each tracing represents a 3–8 hour continuous record.) Thirty cubic centimeters of air were regularly introduced into the balloon-manometer system. The hours since eating, usually 20, and the hours since the last defecation, usually 8–12, were recorded.

RESULTS. The results here submitted represent the findings from nine dogs, 171 tracings, and 451 distinct exercise periods. They are summarized in table 1. Typical graphic records are reproduced in figures 1, 2 and 3.

The purpose of this presentation will be best served if the data are considered under two headings, namely, 1, the normal exercise response; 2, attempts to identify the responsible mechanisms.

1. *The normal exercise response.* Our simplest and basic operation consisted of bringing the blind end of the cecum to the outside through a stab wound in the abdominal wall. After the animal was completely recovered the end of the cecum was opened under local anesthesia. This opening served subsequently for introduction of the balloon and connecting tube into the colon. The modifications of the motility pattern of the standing dog, recorded by this system, when the dog was caused to run on the treadmill we have called the normal exercise response. Dogs 14, 15, and 18, yielded such data. The changes brought on by exercise are of two kinds, *a*, a sharp rise during running, and *b*, a period of reduced activity following the exercise, called by us the "after effect." In figure 1 are reproduced typical tracings showing these effects. Since neither of these effects appear constant in form or regularity a bit of further description is necessary. The "exercise rise" is marked by an abrupt rise in the writing arm of the manometer to three or four times, the height of its level during rest. The first sign of this heightened activity comes from one to five, commonly two

minutes, after the beginning of exercise and subsides again after two to four minutes *whether or not exercise is discontinued in the meantime.* Dog 14, a slight asthenic type, very fond of running, showed this rise in 42 per cent of his running periods, whereas dogs 15 and 18 both notably more muscular and vigorous than dog 14 showed the exercise effect in 80 and 78 per cent, respectively, of their running periods. From our experience with these and other dogs used in the latter portion of this study we were led to expect

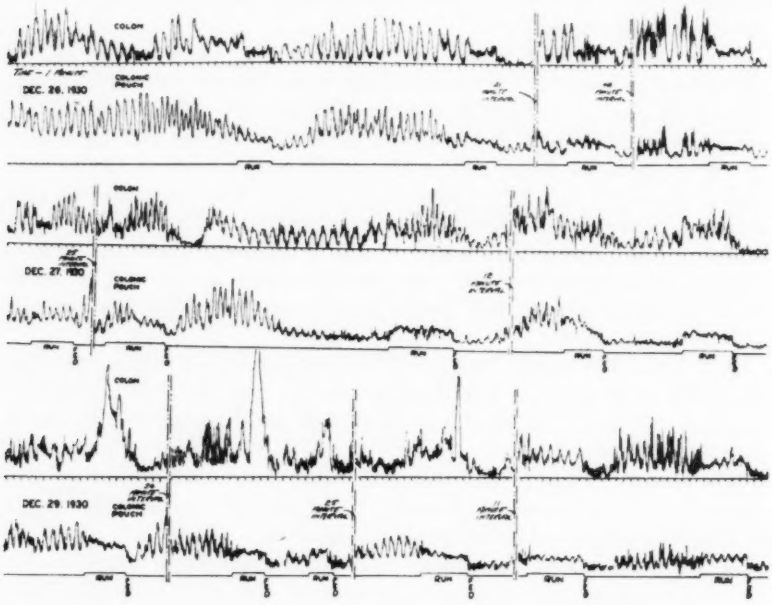

Fig. 3. Three consecutive tracings from the colon and colonic pouch of dog 29. The characteristic exercise rise appears during the running periods of the third tracing, after he had learned to run easily and without fear. The points at which small amounts of chocolate candy were fed after runs, beginning in the second tracing, are indicated. The colonic pouch in no instance records a colonic rise. The tracing of December 29 also illustrates the failure of the exercise rise in the last two of a series of six exercise periods.

this rise as a fairly regular concomitant of exercise whose frequency and intensity varies from dog to dog. Sometimes these rises are accompanied by defecations. There are days, especially when the colon is otherwise quite active, that this exercise rise cannot be elicited. Not infrequently when five or six running periods followed each other at thirty to forty-five minute intervals, the first three or four showed the rise and last two did not, suggesting that the mechanism became fatigued (see figs. 1 and 3).

On rare occasions after a prolonged rest of one and one-half to two hours another rise could be elicited by exercise in these cases.

The exercise period was followed in from 48 to 66 per cent of cases by a period of subnormal motility (see fig. 1) which after from two to sixty minutes again gave way to the normal resting motility picture. Though as a rule the sharpest "exercise rises" were followed by the most marked "after effects," there were times when there was an "after effect" without an "exercise rise," and vice versa.

2. *Attempts to identify the responsible mechanism.* It now became our task to determine if possible the nature of the mechanism responsible for this rather constant and typical modification of colonic motility associated with running. Three types of mechanisms which might modify the action of the intra colonic mechanism suggested themselves: *a*, extrinsic nerves, i.e., the sympathetics or parasympathetics; *b*, direct mechanical or massaging effects of the running movements; *c*, humoral agents such as metabolites or hormones released during exercise. Our first aim was to produce a colon which would not show the exercise effects. To this end we attempted several methods of denervation.

First method. In this method we cut 1st, the colonic branches of the pelvic nerves (nervi erigens) distal to the bladder supply; 2nd, the hypogastric from the inferior mesenteric ganglion; 3rd, any mesenteric nerves which came over the ileocecal junction to join the colon (possible vagal fibers). This method was employed on dogs 14 and 15 which had already served for records of the normal exercise effect (see table 1) and resulted in no perceptible change in the "exercise rise." That dog 14 showed a reduction from 48 to 18 in the percentage of periods showing an "after effect" may be of significance.

Second method. Our second method was identical with the first except that more pains were taken to cut all possible nervous inflow via the mesenteries. The entire mesentery was cut close to the colon leaving it attached only by its vessels each of which was carefully cleaned by dissection. This method was employed on dogs 16 and 17. Both animals showed the "exercise rise" and "after effect" though in only 15 to 22 per cent of cases. Unfortunately these dogs were not first studied as normals. Though in the light of our later findings we may assume that we were partially successful in interfering with the responsible mechanisms, the work with these dogs showed us the inadequacy of such methods of denervation. In several other dogs not recorded here we attempted further denervation by painting the vessel walls with 5 per cent phenol. The dogs did not survive the operation.

Third method. In one dog, namely, dog 18 we cut both pelvic nerves (nervi erigentes) retroperitoneally as they came off the sacral cord and removed, also retroperitoneally, both lumbar sympathetic chains and their

ganglia. This dog showed no change in either the "exercise rise" or the "after effect" following the operation.

Colonic Thiry pouch. To rule out the possibility of extrinsic nerves entering the gut at an inaccessible level and reaching the colon via the gut wall itself we decided to produce a colonic pouch. This was made after the fashion of the Thiry fistula described for the study of the small intestines. Roughly the middle third of the colon was cut out and made into a pouch with its upper end opening through the abdominal wall. The large sympathetic innervation which reaches this middle third through its mesentery was left intact. The remaining portions of the colon were reunited by end to end anastomosis. The regular cecostomy was also produced. This operation was successful on dogs 28 and 29. From them we secured simultaneous records of motility in the colon proper and the colonic pouch. The shortened colon behaved in every respect just as a normal colon whereas the pouch though it otherwise showed entirely normal motility never showed the "exercise rise" (see table 1 and figs. 2 and 3). The "after effect" however was present to the usual extent in both. In dog 28 this pouch was then carefully denervated of its sympathetic supply by our second method described above. This operation had no further effect upon the behavior of either the colon or pouch during or after exercise.

Colon transections. Proceeding on the assumption that the failure of the colonic pouch to show the exercise effects was due to cutting extrinsic nerves which came to it within the gut wall from more distant points of entry, we undertook to determine whether these nerves came from points above or below the region in which we worked. By simply transecting and reuniting the colon at various levels, one at a time, it should be possible to determine the course of such nerves. We therefore proceeded to transect and reunite the colon within an inch or two of the ileocecal juncture (called "high cut" in table 1) or within two inches of the anus (called "low cut" in table 1).

Dog 29 in whom we had previously produced a colonic pouch from the proximal and transverse portions of the colon was already virtually a "high cut" animal. In him we now sectioned and reunited the colon within two inches of the anus. At no time when the balloon was between these two anastomosed points (position A in fig. 2) did exercise induce the characteristic rise. However, when the balloon or part of it was placed distal to the "low cut" (position B in fig. 2) the regular exercise rise was again recorded. Also when the balloon was drawn up very close to the opening of the cecostomy, i.e., proximal to the "high cut" was the exercise rise recorded. The positions of the balloon were established by digital examination per anus before and after running and by marks on the rubber tube. It was easy to palpate by anus both the balloon and the scar marking the

lower cut. In dog 30 we first produced a "high cut" with no appreciable effect on the exercise response (fig. 2). However, when later a "low cut" was produced on the same dog, the "exercise rise" could no longer be elicited while the "after effect" persisted. In dog 31 we produced a "low cut" without destroying the exercise response (fig. 2). Dogs prepared with both "high" and "low" sections invariably evidenced difficulty in defecation. Upon being turned out they would assume extreme defecating positions which they would maintain for thirty to forty-five minutes until put back in their cages. By forceful contraction of abdominal muscles they expelled more or less of a dark, wet fecal mass. One dog sacrificed after being in this condition for four months showed a colon distended to three times its normal diameter with a black, foul smelling, soft mass. Another dog killed three weeks after the second cut showed a similar condition though not so far advanced. These conditions explained to us the reason for the very high percentage of fatalities which we had experienced in making these sections, especially when we produced the "low cut" first. Acting upon this suggestion we began in our last dog to withdraw daily under negative pressure the fecal matter which accumulated within a few days after the operation and were successful in saving him.

Psychic factors. Not of least importance in throwing light on the mechanism responsible for the exercise pictures is our experience with dog 29 in whom we had produced a colonic pouch as well as cecostomy. This dog long resisted all of our ordinary methods for teaching dogs to run on the treadmill. Finally he learned, but always ran with tail between his legs and other signs of fear. Under these conditions he failed to show any "exercise rise" in the colon. Fortunately the dog enjoyed chocolate candy, so we began feeding him a bit right after each run. On the third day following the initiation of this practice he ran with evident lack of fear and showed "exercise rises" as other dogs. In figure 3 are reproduced the three successive tracings which mark this transition in his behavior. This animal merely showed in accentuated form an observation we had made on most of our dogs, namely, that during the first two or three trials the colon seldom showed an exercise rise. All of the other dogs, however, grew accustomed to the regime on the second or third day of testing, without special encouragement.

Discussion of results. From x-ray observations on patients Hertz (1907) described mass movements of the colon occurring several times daily. In 1913 Hertz and Newton found that these mass movements may be brought on by eating a small meal and ascribed the association to a gastro-colic reflex. They state, also, that these movements may be influenced by the mind. Simultaneously with such mass movements their patients reported an urge to defecate and in some cases defecation took place. Similar movements have been observed by Holzknecht (1909),

Barclay (1912), and Case (1915). We believe that the exercise rise observed by us and often accompanied by defecation is in nature very similar to these mass movements observed in man. That the general somatic stimulation accompanying running should increase the motility of the colon might be anticipated from the work of Lehmann (1913) who found that stimulation of the central end of the vagus and somatic sensory nerves (sciatic) in dogs exerts a motor influence on the large intestine. Concerning the mechanisms by which the described exercise changes are produced our data are not without positive suggestions. The direct mechanical massaging action of the exercise movements of the abdominal wall is ruled out as the causative stimulus by the fact that the colonic pouch in dogs 28 and 29, though subject to equal stimulation from this source never showed the exercise rise while the intact colon immediately beside it showed the rise. Further, the absence of this rise in the colon of dog 29, figure 3, when he ran with fear of the treadmill and its presence after he became accustomed to running cannot be attributed to a difference in this form of stimulation. Exercise metabolites, hormones, or changes in body temperature as possible causes of the "exercise rise" must be ruled out by the same reasoning. Their possible relation to the "after effect" is, however, not ruled out as will become clear a little later.

The absence of the exercise rise in the colonic pouch, in the colon with "high" and "low cuts," and in the frightened dog can, to our mind, be interpreted only as indicating that an extrinsic nervous mechanism is responsible for this rise. All authorities are agreed that the colon is supplied with sympathetic and with parasympathetic fibers. The former, the hypogastric, enter the middle third of the colon reaching it via the mesentery. To this system is usually ascribed an inhibiting function. Kuntz (1929) states that in some instances the inhibition elicited by stimulation of the splanchnic or hypogastric nerves is preceded by contraction. The fact that this nerve was left undisturbed in the pouches which failed to show the "exercise rise," however, eliminates the sympathetics from responsibility for the rise. The pelvic nerve carries the lumbosacral outflow of parasympathetic fibers to the colon. Its many colonic branches enter the colon wall very close to the anus. There is general agreement among writers that these fibers augment colonic tone and motility. By transecting the colon two inches above the anus as we did in our "low cut" dog 31 we undoubtedly interrupted all fibers passing forward in the colon from this point of entrance. That this dog showed the exercise rise (the rise in this dog was never as high as in normal dogs or dogs with a "high cut") suggests that there must be another source of parasympathetic stimulation. The complete loss of the exercise rise in portions of the colon between both high and low sections and its presence in the portions of the colon *above* the "high cut" and *below* the "low cut" as observed by us in dogs 29 and 30 forces us

to the conclusion that there is a second parasympathetic inflow, probably vagal, at the upper end of the colon. Boehm (1913) showed increased motility in the colon of cats and rabbits upon stimulating the vagus under the diaphragm. His observation that repeated stimulation of either the pelvic nerve or the vagus resulted ultimately in failure to elicit a motor response is remarkably like our observation that the exercise rise fails to appear after repeated trials. Numerous workers, among them Bayliss and Starling (1900), Elliott and Barclay Smith (1904), Klee (1912) and Gaskell (1916) are of the opinion that vagus fibers do not supply any portion of the large intestine. Alvarez (1929) writing on this point suggests that different results would probably have been secured with balloons recording in *unanesthetized* animals. Such conditions prevailed in our experiments.

In the light of these interpretations we ascribe our failure to prepare colons free from the "exercise rise" by our first, second and third methods of denervation as due to missing either part of the pelvic nerve or the vagus branches or both. The low percentage of exercise rises observed in dogs 16 and 17 may indicate a fairly close approximation to complete denervation by these methods.

Whether during exercise there is an unusual outflow of impulses over the parasympathetics which initiates the exercise rise or whether the parasympathetics merely supply to the colon continually a higher tonic level which makes it more sensitive to a local, chemical or mechanical stimulus supplied by exercise, may to some still remain an unsettled question. The fact that both the colonic pouches and the sections of colon between the "high" and "low cuts" showed approximately normal tone and activity when the balloon was inflated against their walls, appears to us to offer support to the first of these alternatives.

Our data are not as convincing in pointing out a mechanism for the "after effect" of subnormal motility. Since it is found in portions of the colon such as our pouches which are clearly free from parasympathetics, the possibility of fatigue of parasympathetic tone resulting from hyper-stimulation during exercise need not be considered. The presence of the "after effect" in all our preparations in which the sympathetics were cut would more convincingly rule out a direct inhibitory effect of this system if we could be sure of complete denervation. Plant and Miller (1926) have shown the practical difficulties involved in such procedures. Whereas in dog 14 the percentage of runs showing "after effects" is reduced after attempts to denervate, it remains unchanged or actually increases following similar operations in dogs 15, 18, and 28. If we may assume that the majority of fibers were cut in our attempts to denervate, then this failure to get some consistent reduction in the extent of the "after effect" would suggest that other mechanisms are probably responsible. From the work of Van

Liere and Crisler (1930) and Crisler and Van Liere (1931) which indicates that lowered oxygen tensions reduce stomach motility, the possibility of a local anoxemia due to vasomotor changes suggests itself in this connection. The intense contraction of the colon wall during exercise may itself help induce such an anoxemic condition.

SUMMARY

1. The effects of short periods of strenuous treadmill running on the motility of the colon of dogs, completely recovered from appropriate surgical intervention were recorded by the balloon method in a series of nine dogs.

2. During an exercise period there is in a majority of cases a marked rise in colonic tone and motility. This rise is *not* coextensive with the running period. It begins usually from one to three minutes after the start of exercise and recedes after a few minutes without reference to the duration of exercise.

3. From studies following a variety of denervation procedures it is concluded that the appearance of this rise depends on the intactness of at least one of two parasympathetic inflows, namely, either the pelvic nerve (nervus erigens) from below or fibers, probably vagal, entering the colon over the ileocecal juncture from above.

4. The rise did not appear in a dog who ran with evident fear of the treadmill until after he had been conditioned to run without this emotion.

5. Following the exercise period there is usually a period of subnormal activity of varying length—sometimes as long as forty-five minutes.

6. Our data are not adequate to determine the nature of the mechanism responsible for the "after effect." The weight of evidence appears to favor a non-nervous mechanism.

In its early exploratory stages Mr. Oscar Mertins threw himself wholeheartedly into the conduct of this study. What added insight would have been brought to bear on the solution of this problem had his relation to it not been cut short by his untimely death, August 7, 1928, will remain for always unknown.

BIBLIOGRAPHY

ALVAREZ, W. C. 1929. The mechanics of the digestive tract. Paul B. Hoeber, N. Y., p. 240.

BARCLAY, A. E. 1912. Arch. Roentg. Ray, xvi, 422.

BAYLISS, W. M. AND E. H. STARLING. 1900. Journ. Physiol., xxvi, 107.

BOEHM, G. 1913. Arch. f. exper. Path. und Pharm., lxxii, 1.

CASE, J. T. 1915. Arch. Roentg. Ray, xix, 375.

CRISLER, G. AND E. J. VAN LIERE. 1931. This Journal, xcvii, 516.

ELLIOTT, T. R. AND E. BARCLAY-SMITH. 1904. Journ. Physiol., xxxi, 272.

GASKELL, W. H. 1916. The involuntary nervous system. Longmans, Green & Co., London, 52.
HERTZ, A. F. 1907. Guy's Hosp. Rept., lxi, 389.
HERTZ, A. F. AND A. NEWTON. 1913. Journ. Physiol., xlvii, 57.
HOLZKNECKT, G. 1909. Münch. med. Wochenschr., lvi, 2401.
IVY, A. C. AND C. A. VLOEDMAN. 1923. This Journal, lxvi, 141.
KLEE, P. 1912. Pflüger's Arch., cxlv, 557.
KUNTZ. 1929. Autonomic nervous system. Lea and Febiger, Philadelphia, p. 218.
LEHMANN, A. 1913. Pflüger's Arch., cxlix, 413.
PLANT, O. H. AND G. H. MILLER. 1926. Journ. Pharm. Exper. Therap., xxvii, 149.
PUCH, J. 1794. A treatise of the science of muscular action. London.
STEINHAUS, A. H AND V. R. DEYOUNG. 1931. This Journal, xcvii, 564.
STEINHAUS, A. H, C. B. STANWOOD, J. AND F. SLATER, G. SCHEUCHENPFLUG. 1928. Proc. Soc. Exper. Biol. and Med., xxv, 599.
VAN LIERE, E. J. AND G. CRISLER. 1930. This Journal, xciii, 267.

A DELIMITATION OF THE CENTRAL NERVOUS MECHANISM INVOLVED IN REFLEX HYPERGLYCEMIA[1]

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Claude Bernard in 1858 demonstrated very clearly, by means of his experiments upon sugar puncture, the possibility of a nervous control of carbohydate mobilization. Since that time the piqûre method has often been employed in an effort to locate some central point responsible for the hyperglycemia and glycosuria but with questionable success. Brugsch, Dresel, and Lewy (1920; 1921), using this method, concluded that there were two sugar-centers present in the floor of the fourth ventricle. If one of these was irritated there resulted a lowering of the blood sugar, presumably because of an increased activity of the pancreas; if the other center was punctured the blood sugar rose. This and other efforts to localize a sugar-center in the brain by means of piqûre have been criticized by Hiller and Tannenbaum (1929), who point out that any injury to the brain and even the manipulations preliminary to puncturing may cause a transient hyperglycemia quite comparable to that induced by puncturing the region which is supposed to contain the sugar-center.

The work of Griffith (1923) upon the peripheral factors involved in reflex hyperglycemia showed that the organs chiefly responsible for the reflex variations were the liver and the adrenal glands. More recently Simnitzky, Wischnewsky, and Satwornitzkaja (1930) have likewise shown that the adrenal glands are largely responsible for the reflex hyperglycemia and also that the reduction of the blood sugar to a normal level is due to an activation of the pancreas. As a method which can be employed in a study of the factors involved in carbohydrate mobilization this reflex method is, as Griffith has pointed out, far superior to the rather unphysiological procedure of puncturing the floor of the fourth ventricle. Considering anew the possibility of the existence of a bulbar control of carbohydrate mobilization it was suggested by Bard (1929) that "another method of approach to this problem would be to investigate the possibility of inducing reflex rises in blood sugar with various parts of the central nervous system excluded,——." The results reported in this paper were obtained during such an investigation.

[1] A preliminary report of the work reported in this paper appeared in Proc. Soc. Exp. Biol. and Med., 1931, xxviii, 524.

METHODS AND RESULTS. Well-nourished cats which had been in the laboratory for some time were used for this work. To make certain that the digestion of food was not occurring at the time of the experiment and to insure the most uniform sugar storage and blood sugar level possible the animals were not fed for twelve or eighteen hours before being used.

For a comparison of the relative abilities of intact, thalamic, decerebrate, and spinal animals to give reflex hyperglycemias it is obvious that an anesthetic must be employed in every case. The anesthetic used in most of these experiments was sodium barbital. Chloralose has been employed quite generally in studies of reflex blood sugar rises and since it is known that it does not change the amount of sugar in the blood (Griffith, 1923), some experiments were performed under chloralose. It was found, however, that chloralose affects the respiratory mechanism in such a way that the animals seldom breathe well after they have been decerebrated. Sodium barbital, on the other hand, is an excellent anesthetic for any type of cerebral operation and although the reflex rises are never as great as those obtained when chloralose is used, it seems to have no direct effect upon the blood sugar level. The fact that cats under sodium barbital maintained a constant normal blood sugar level for five or six hours when undisturbed and when stimulated show a reflex hyperglycemia which is in every way comparable, except in magnitude, to that obtained in an animal under chloralose, is ample evidence that sodium barbital is equally reliable for blood sugar studies. Chloralose (0.1 gram per kgm. of body weight), when used, was dissolved in about 40 cc. of milk and given by mouth. In the first experiments in which sodium barbital was used the anesthetic was injected intraperitoneally (0.3 gram per kgm.), but it was soon found that by etherizing the animals, exposing the left femoral vein, and injecting the anesthetic intravenously (0.275–0.28 gram per kgm.) with 15 to 20 cc. of warm saline solution the animals could be more quickly and more easily anesthetized and a more uniform degree of anesthesia could be obtained.

Samples of circulating blood (0.5 cc.) were collected by means of a small syringe from a cannula tied into the right femoral artery just distal to its saphenous branch. The method used for the blood sugar assay was that of Folin (1929). Each sample was treated in exactly the same way. Two or three blood samples were taken at half-hour intervals to ascertain the blood sugar level before stimulation. After the third sample had been taken the right brachial nerve was exposed very carefully but as quickly as possible and Sherrington shielded electrodes attached. Stimulation was with a tetanizing current from the secondary coil of a Harvard inductorium and the current was of the same intensity in all experiments. The stimulation was interrupted, being on 10 seconds, off 5, on 10, etc., for a period of three minutes. Blood samples were drawn one minute, 10 minutes, 30 minutes, one hour, and two hours after the stimulation. Records of

arterial pressure and heart rate were taken throughout each experiment in order to ascertain whether or not the animal was in good condition. Temperature, as shown by a rectal thermometer, was maintained as nearly constant and normal as possible.

In order to detect any abnormality or deficiency of the reflex blood sugar rise in animals from which portions of the central nervous system had been removed, it was necessary to learn the height and duration of the reflex rise

TABLE 1

Control experiments

SODIUM BARBITAL GIVEN INTRAPERITONEALLY (0.3 GRAM PER KGM.)										SODIUM BARBITAL GIVEN INTRAVENOUSLY (0.28 GRAM PER KGM.)									
	Blood sugar			Heart rate			Blood pressure				Blood sugar			Heart rate			Blood pressure		
Experiment number	Blood sample before stimulation	Rise in mgm. per 100 cc.	Rise in per cent	Rate before stimulation	Rise in beats per minute	Rise in per cent	Before stimulation in mm. of Hg	Rise in mm. of Hg	Rise in per cent	Experiment number	Blood sample before stimulation	Rise in mgm. per 100 cc.	Rise in per cent	Rate before stimulation	Rise in beats per minute	Rise in per cent	Before stimulation in mm. of Hg	Rise in mm. of Hg	Rise in per cent
5	95	95	100	210	45	21	130	26	20	19	190	20	10	252	18	7	146	50	34
	140	45	32	210	15	7	105	25	23	20	167	15	8	260	16	6	106	15	14
	160	45	28	240	15	6	120	35	29	21	143	11	7	197	40	20	115	45	39
										22	139	10	7	240	20	8	147	43	29
10	187	25	13	267	32	12	140	20	13	23	123	29	24	200	36	18	126	14	11
13	110	45	41	135	35	26	130	50	37	24	143	17	12	248	20	8	157	37	23
	137	19	13	145	40	27	125	85	68	25	135	28	21	220	23	10	114	25	22
14	140	10	7	160	25	15	105	15	14	26	190	32	17	260	30	12	138	32	23
	100	7	7	185	27	14	110	60	54	60	111	12	11	220	20	9	135	30	22
17	132	10	8	208	12	5	112	10	8	61	95	27	28	145	40	27	120	45	37
18	161	6	4	160	35	21	95	45	5										
	163	17	10	211	15	7	120	15	12										
Aver....	138	29	21	194	27	14	117	34	29		144	20	14	224	26	17	130	34	26

in animals which had been anesthetized and stimulated in the same way, but in which the nervous system remained intact.

Control experiments. Twenty-six control experiments were performed; ten under chloralose anesthesia, six under sodium barbital injected intraperitoneally, and ten under sodium barbital intravenously. The results of these experiments are recorded in tables 1 and 2.

In these experiments as soon as anesthesia was sufficiently deep a tracheal

cannula was inserted and the carotid arteries ligated in the neck. The right and left femoral arteries were then carefully exposed, a pressure cannula was tied into the left, and a small glass cannula, by means of which blood samples could be obtained, was inserted in the right femoral artery. Because the rise in blood sugar concentration caused by even such slight operative procedures as these is considerable, the first blood pressure records and blood samples were not taken until at least an hour had elapsed.

Thalamic animals. Although Morita (1915) stated that the center controlling carbohydrate mobilization and reflex hyperglycemia was not in the hemispheres or the forebrain it seemed best to confirm this statement if possible. Consequently a series of thalamic animals was prepared. The

TABLE 2

Control experiments

Chloralose given by mouth (0.1 gram per kgm. in 40 cc. of milk)

EXPERIMENT NUMBER	BLOOD SUGAR			HEART RATE			BLOOD PRESSURE		
	Blood sample before stimulation	Rise in mgm. per 100 cc.	Rise in per cent	Rate before stimulation	Rise in beats per minute	Rise in per cent	Before stimulation in mm. of Hg	Rise in mm. of Hg	Rise in per cent
27	115	18	16	75	75	100	190	40	21
30	100	15	15	170	25	14	110	30	27
36	90	40	44	215	0	0	160	30	18
38	115	45	39	255	30	11	175	40	22
40	205	17	9	210	20	9	155	40	25
49	85	27	32	160	40	25	170	20	11
50	75	8	10	130	28	21	125	15	11
62	138	18	13	208	12	6	140	20	14
64	127	14	11	164	51	31	87	40	45
65	65	15	23	176	36	21	170	30	18
Averages...	111	22	21	176	32	18	148	31	21

ten animals used were anesthetized by intravenous injections of sodium barbital. After the tracheal cannula had been inserted and the carotids tied, the brain was exposed and the cerebral hemispheres removed by a method described by Bard (1928). A diagonal cut was then made from a point a few millimeters ahead of the anterior corpora quadrigemina to a point a millimeter or two anterior to the optic chiasma, thus removing the cerebral hemispheres, the corpora striata, and the cranial part of the thalamus. Little blood was lost in any case and the respiration, heart rate and arterial pressure indicated that all the animals were in excellent condition after the operation. The first blood samples were not taken until one hour after the operation but from then on the procedure was the same as in the control experiments.

The results of this series of experiments are summarized in table 3. The reflex blood sugar rise was of the same order of magnitude as in the animals with central nervous system intact. If the average rise (26 mgm. per cent) in this group of ten animals anesthetized with sodium barbital given intravenously be compared with the average rise (20 mgm. per cent) of the ten control animals similarly anesthetized, it is seen that the thalamic preparation yields, on the average, a somewhat greater reflex hyperglycemia. On the other hand, if this rise be compared with the average rise (24 mgm. per cent) of all twenty-six intact animals, variously narcotized, it appears that the thalamic cat gives practically the same reflex rise. The former comparison seems the more valid one. The

TABLE 3

Thalamic animals

Sodium barbital given intravenously (0.28 gram per kgm.)

EXPERIMENT NUMBER	BLOOD SUGAR			HEART RATE			BLOOD PRESSURE		
	Blood sample before stimulation	Rise in mgm. per 100 cc.	Rise in per cent	Rate before stimulation	Rise in beats per minute	Rise in per cent	Before stimulation in mm. of Hg	Rise in mm. of Hg	Rise in per cent
75	177	27	15	180	10	6	130	37	27
76	177	18	10	243	17	7	100	53	53
77	200	20	10	238	20	8	140	65	46
78	136	34	25	240	20	8	150	27	18
79	104	18	17	220	34	16	140	58	41
81	184	53	28	264	28	11	130	22	17
82	160	23	14	200	60	30	90	50	55
85	97	23	24	164	60	36	110	38	34
86	125	21	17	232	12	5	138	12	9
87	108	21	19	212	32	15	80	20	25
Averages...	147	26	18	219	29	13	121	33	27

thalamic animals, unless deeply anesthetized, showed occasional periods of spontaneous activity representing in modified form the pseudaffective phenomena which are so characteristic of unanesthetized thalamic animals (Cannon and Britton, 1925; Bulatao and Cannon, 1925; Bard, 1928). In view of the fact, stated in the next section, that ten decerebrate animals exhibiting no trace of pseudaffective activity and under the same type of anesthesia gave an average rise (27 mgm. per cent) which is essentially the same as that of the thalamic cats, it is improbable that an overactive diencephalon is responsible for the difference between the thalamic and control reflex hyperglycemias.

Decerebrate animals. Twenty-six decerebrate animals were prepared in which the brain stem had been sectioned at the level of the anterior corpora

quadrigemina. As in the series of control experiments ten operations were performed with the use of chloralose as the anesthetic and six with sodium barbital which had been injected intraperitoneally. Since there was always a difference in the basal level of the blood sugar, in the degree of anesthesia, and probably in the sugar storage of each animal, an attempt was made to eliminate the individual differences by taking the reflex rise of an intact animal, then decerebrating it and again measuring the reflex rise obtained by the same stimulation of the same nerve. Ten experiments of this nature were performed upon animals which had been anesthetized by intravenously injected sodium barbital (compare tables 1 and 4). The following protocol indicates the usual procedure.

Experiment 23. Control experiment combined with a decerebration experiment. Weight 3.12 kgm. Male.

2:00 p.m.	Animal tied on animal board and etherized.
2:10	Tracheal cannula inserted.
2:15	Both carotid arteries ligated in neck.
2:30–2:40	Injected 17.5 cc. of a 5 per cent solution of sodium barbital and 20 cc. of a 0.9 per cent warm saline solution into the left femoral vein.
2:55	Cannula for collecting blood samples placed in the right femoral artery just distal to its saphenous branch.
3:25	Blood pressure cannula in left femoral artery.
3:30	Blood sample 1–125 mgm. per 100 cc. of blood.
3:59	Blood sample 2–120 mgm. per 100 cc. of blood.
4:29	Blood sample 3–129 mgm. per 100 cc. of blood.
4:33–4:40	Right brachial nerve exposed and electrodes adjusted.
4:57–5:00	Stimulation of the nerve. Considerable reflex activity aroused.
5:01	Blood sample 4–152 mgm. per 100 cc. of blood.
5:13	Blood sample 5–134 mgm. per 100 cc. of blood.
5:46	Blood sample 6–125 mgm. per 100 cc. of blood.
6:00	Skull exposed.
6:10	Decerebration completed. Very little blood lost. Breathing regular and normal. Heart strong.
6:40	Animal tied down again.
6:43	40 cc. warm saline injected subcutaneously.
6:50	Blood sample 7–133 mgm. per 100 cc. of blood.
7:18	Blood sample 8–120 mgm. per 100 cc. of blood.
7:20–7:27	Electrodes again adjusted.
7:27–7:30	Stimulation.
7:31	Blood sample 9–148 mgm. per 100 cc. of blood.
7:42	Blood sample 10–125 mgm. per 100 cc. of blood.
8:10	Blood sample 11–118 mgm. per 100 cc. of blood.
8:44	Blood sample 12–125 mgm. per 100 cc. of blood.

Decerebration was performed by exposing the brain dorsally, removing the hemispheres, and making a section through the anterior corpora quadrigemina which extended ventrally to a point a few millimeters cranial to the pons. The carotids had previously been ligated and bleeding was con-

TABLE 4

Decerebrate animals

	SODIUM BARBITAL GIVEN INTRAPERITONEALLY (0.3 GRAM PER KGM.)										SODIUM BARBITAL GIVEN INTRAVENOUSLY (0.28 GRAM PER KGM.)									
	Blood sugar			Heart rate			Blood pressure				Blood sugar			Heart rate			Blood pressure			
Experiment number	Blood sample before stimulation	Rise in mgm. per 100 cc.	Rise in per cent	Rate before stimulation	Rise in beats per minute	Rise in per cent	Before stimulation in mm. of Hg	Rise in mm. of Hg	Rise in per cent	Experiment number	Blood sample before stimulation	Rise in mgm. per 100 cc.	Rise in per cent	Rate before stimulation	Rise in beats per minute	Rise in per cent	Before stimulation in mm. of Hg	Rise in mm. of Hg	Rise in per cent
6	200	40	20	255	5	2	130	15	11	19	196	14	7	256	4	1	70	17	24
8	152	26	17	227	45	20	130	57	43	20	131	20	15	260	27	10	93	27	29
9	83	14	16	188	37	20	90	40	44	21	105	21	20	224	25	11	120	50	41
11	93	58	68	170	10	5	105	55	54	22	174	7	4	280	8	3	87	10	11
12	148	43	44	260	35	13	100	23	23	23	120	28	23	211	30	14	88	80	91
16	113	66	59	213	21	9	80	23	28	24	176	45	26	252	15	6	100	45	45
										25	191	45	24	240	20	8	128	42	33
										26	200	38	19	284	0	0	80	22	27
										60	111	31	28	220	15	7	100	40	40
										61	129	16	12	205	5	2	100	45	45
Aver....	132	41	31	219	26	12	106	36	33		153	27	17	243	15	6	97	38	39

TABLE 5

Decerebrate animals

Chloralose given by mouth (0.1 gram per kgm. in 40 cc. of milk)

EXPERIMENT NUMBER	BLOOD SUGAR			HEART RATE			BLOOD PRESSURE		
	Blood sample before stimulation	Rise in mgm. per 100 cc.	Rise in per cent	Rate before stimulation	Rise in beats per minute	Rise in per cent	Before stimulation in mm. of Hg	Rise in mm. of Hg	Rise in per cent
44	235	50	21	230	50	22	85	30	35
47	115	10	9	135	30	22	66	8	12
52	95	16	17	240	30	12	160	74	46
53	85	20	24	136	24	17	110	95	86
64	78	7	10	195	10	5	110	60	54
65	86	14	16	204	8	4	140	30	21
66	81	14	17	130	10	8	100	33	33
68	133	31	23	232	16	7	162	18	11
69	119	21	18	228	36	16	115	89	77
70	76	13	17	152	12	8	110	50	45
Averages...	129	20	17	188	23	13	116	49	42

trolled by compression of the vertebrals. Usually some blood was lost, however, and in order to aid the animal in recovering its normal blood volume 20 cc. of warm saline solution were always injected subcutaneously. The experiment was not continued unless the respiration was unimpaired. The first blood sample was taken approximately one hour after the operation had been completed.

The results, which are presented in tables 4 and 5, show quite conclusively that the reflex blood sugar rises obtained from animals decerebrated at this level are not essentially different from those shown by the control animals.

TABLE 6

Decapitate animals

CHLORALOSE GIVEN BY MOUTH (0.1 GRAM PER KGM. IN 40 CC. OF MILK)										ETHER ANESTHESIA UNTIL OPERATION WAS COMPLETED									
	Blood sugar			Heart rate			Blood pressure				Blood sugar			Heart rate			Blood pressure		
Experiment number	Blood sample before stimulation	Blood sample after stimulation	Change in per cent	Rate before stimulation	Rise in beats per minute	Rise in per cent	Before stimulation in mm. of Hg	Rise in mm. of Hg	Rise in per cent	Experiment number	Blood sample before stimulation	Blood sample after stimulation	Change in per cent	Rate before stimulation	Rise in beats per minute	Rise in per cent	Before stimulation in mm. of Hg	Rise in mm. of Hg	Rise in per cent
28	153	143	−6	165	0	0	30	2	7	33	175	173	−1	184	39	21	40	14	35
29	52	59	13	155	5	3	30	5	17	34	148	135	−9	205	18	9	30	10	33
31	180	177	−2	160	5	3	40	7	18	35	250	227	−9	200	25	13	41	15	37
32	118	123	4	150	8	5	42	8	19	39	157	142	−10	180	64	36	80	10	13
36	204	188	−8	168	4	3	45	6	13	48	160	90	−43	136	19	14	60	5	8
37	152	143	−6	145	15	10	30	5	17										
40	110	96	−12	177	8	5	30	3	10										
42	150	132	−12	140	5	4	33	4	12										
49	160	170	6	220	20	9	50	34	68										
50	154	129	−17	184	21	11	45	4	9										
Aver....	143	136	−5	166	9	5	38	8	20		178	153	−14	181	33	18	50	11	21

On the basis of these findings it can be stated that the central mechanism concerned in reflex hyperglycemia is located below the level of the anterior colliculi.

Decapitate animals. Fifteen decapitate animals were studied. In ten experiments chloralose was employed but in the remaining five ether was used until decapitation was completed. At the time of stimulation, therefore, these latter animals were virtually unanesthetized. The preparations remained in good condition for as long as twelve to eighteen hours if undisturbed, but rarely over ten hours if stimulated. Stimulation induced considerable activity, especially in the animals which had been decapitated

under ether, and this was associated with cardiac acceleration and increased arterial pressure.

This work confirms that of Mellanby (1926) who found that sensory stimulation of spinal cats, prepared by Langley's method of injecting starch grains into the cerebral circulation, did not produce reflex hyperglycemia. After decapitation the blood sugar slowly but steadily decreased, a result comparable to that mentioned by Olmsted and Coulthard (1927). Asphyxiation caused a considerable rise in the sugar content of the blood but stimulation of an afferent nerve had no effect on blood sugar. The question of spinal shock and its duration is a necessary consideration and though reflex cardiac acceleration and increases in arterial pressure were

TABLE 7

Section through medulla oblongata from posterior border of corpora quadrigemina to the posterior border of the pons

CHLORALOSE GIVEN BY MOUTH (0.1 GRAM PER KGM. IN 40 CC. OF MILK)										SODIUM BARBITAL GIVEN INTRAVENOUSLY (0.28 GRAM PER KGM.)									
	Blood sugar			Heart rate			Blood pressure				Blood sugar			Heart rate			Blood pressure		
Experiment number	Blood sample before stimulation	Rise in mgm. per 100 cc	Rise in per cent	Rate before stimulation	Rise in beats per minute	Rise in per cent	Before stimulation in mm. of Hg	Rise in mm. of Hg	Rise in per cent	Experiment number	Blood sample before stimulation	Rise in mgm. per 100 cc.	Rise in per cent	Rate before stimulation	Rise in beats per minute	Rise in per cent	Before stimulation in mm. of Hg	Rise in mm. of Hg	Rise in per cent
54	110	20	18	212	23	11	93	17	18	71	136	18	13	216	20	9	120	16	13
55	98	5	5	250	15	6	90	4	4	72	223	17	8	288	16	6	92	30	33
56	165	62	38	263	15	6	90	20	22	73	200	12	6	216	32	15	96	38	39
57	110	39	35	210	25	12	80	15	19	76	200	20	10	290	10	3	93	14	15
										77	243	34	14	270	20	8	123	27	21
										84	154	28	18	264	24	9	84	26	31
Aver....	121	32	26	234	20	9	88	14	15		192	22	11	257	20	8	101	25	25

obtained, there is a slight possibility that the experiments were not of sufficient duration to permit the recovery of the mechanism controlling carbohydrate mobilization, if such a mechanism does exist. In a few cases a brief retardation of the fall or even a rise of a few milligrams was obtained, but it can be safely said that under the conditions of these experiments the typical reflex hyperglycemia is not obtainable (see table 6).

Further delimitation of the reflex blood sugar center. The experiments described above indicated that the reflex mechanism controlling carbohydrate mobilization is located in the medulla oblongata. In order to find the part of the medulla responsible for this phenomenon sections were made through various regions and the reflex hyperglycemia measured.

In this work the method employed by Cannon and Rapport (1921) in locating the center responsible for the reflex activity of the adrenal glands was followed. The cerebral hemispheres were ablated and the tentorium was carefully removed. Using a sharp knife sections were made through the brain stem just back of the midbrain. By making cuts diagonally backward the anterior part of the medulla could be easily removed as far back as the posterior border of the pons. Although the carotids were ligated and the vertebral arteries compressed, considerable bleeding usually resulted; therefore, a subcutaneous injection of 20 cc. of warm saline solution was given as soon as possible. Such animals breathed but generally respiration was somewhat insufficient and for this reason the experiments were continued under artificial respiration to insure adequate ventilation in all instances. The breathing, however, was perfectly adequate to maintain life for considerable periods of time and frequently animals were

TABLE 8

Sections through medulla from the point of union of the cerebellum and the brachium pontis to the posterior border of the trapezium

Sodium barbital given intravenously (0.28 gram per kgm.)

EXPERIMENT NUMBER	BLOOD SUGAR			HEART RATE			BLOOD PRESSURE		
	Blood sample before stimulation	Blood sample after stimulation	Change in per cent	Rate before stimulation	Rise in beats per minute	Rise in per cent	Before stimulation in mm. of Hg	Rise in mm. of Hg	Rise in per cent
75	199	198	−0.5	187	25	13	75	20	26
82	145	151	4	244	6	2	75	35	46
88	100	105	5	148	12	8	65	5	8
89	132	140	6	152	8	5	80	15	18
Averages...	144	149	3.8	183	13	7	74	18	24

able to keep themselves alive and fairly normal, as regards arterial pressure and heart rate, for as long as three hours after the artificial respiration had been discontinued at the end of the experiment. During the experiments arterial pressure was normal and reflex changes in both arterial pressure and heart rate were similar to those obtained in decerebrate animals. Essentially the same reflex rises in blood sugar were obtained as in the other preparations giving positive results (see table 7).

If, however, the section was made further back, from the origin of the brachium pontis in the cerebellum, on the dorsal side, to the posterior border of the trapezoid body, on the ventral side; or if the cerebellum was removed and a section made from the posterior border of the brachium pontis to the posterior border of the pons, no significant reflex blood sugar rise was obtained, and the animals approximated decapitate animals in this respect. These results are to be found in table 8.

DISCUSSION. As a result of his efforts to find the cause of the puncture diabetes, Claude Bernard discovered that the phenomenon could not be produced if the spinal cord were severed anterior to the origin of the splanchnic nerves or if the splanchnic nerves alone were cut. Eckhard (1869) demonstrated conclusively that splanchnic section made piqûre ineffective and Kahn (1917) found that removal of the adrenals prevented puncture diabetes. Carrasco-Formiguera (1922) by showing that sugar puncture increased the amount of adrenin in the blood, furnished additional and very satisfactory proof that the splanchnic nerves and the adrenal glands were largely responsible for the rise in blood sugar due to puncturing the floor of the fourth ventricle.

Griffith (1923) has demonstrated that stimulation of an afferent nerve raises the blood sugar chiefly by activating the adrenal glands. Therefore any center controlling the amount of adrenalin discharged into the blood might be regarded as a center influencing carbohydrate mobilization. Definite information is at hand regarding the central control of medulliadrenal secretion. In 1912 Elliott presented evidence showing that the loss of adrenalin from the glands which follows afferent stimulation or cerebral injury is mediated by a center close to the bulbar vasomotor center. Further, Cannon and Rapport were able more definitely to locate the reflex center by the method of successive transections of the brain stem and they found it near the upper edge of the fourth ventricle just back of the inferior colliculi. Thus the piqûre experiments and the study of reflex excitation of the adrenals seem to furnish satisfactory proof for the existence of a center in the upper part of the bulb capable of influencing medulliadrenal secretion.

In the present series of experiments reflex hyperglycemias fully as great as those produced in animals with intact central nervous systems were evoked after the medulla had been transected below the locus of the adrenal center of Cannon and Rapport. From this it would appear that the central mechanism involved in reflex hyperglycemia is not identical with the reflexly excitable adrenal center. It is worth noting that in Griffith's experiments denervation of the liver reduced the reflex rises in blood sugar on afferent stimulation. Although the percentages obtained in my experiments were somewhat lower than those he was able to produce when both the adrenals and the liver were active, they were slightly higher than the percentages he obtained after the adrenals were inactivated. Accordingly my rises may have been due chiefly to a reflex excitation of the nerves to the liver. But the elucidation of this point must await a further experimental analysis.

The fact that reflex rises in blood sugar equal to those produced in animals with intact central nervous systems are obtained after extirpation of the forebrain, midbrain, and the anterior part of the medulla oblongata does not indicate that higher centers have no ability to control carbohydrate

mobilization. The diencephalic center which is responsible for decorticate sham rage (Bard, 1928) certainly has the ability to bring about the mobilization of carbohydrate stores (Bulatao and Cannon, 1925). There is other excellent evidence that the hypothalamus can exert some influence upon carbohydrate mobilization. Sachs and MacDonald (1925), Bailey and Bremer (1921), Camus and Roussy (1920), and Camus, Gournay, and Le-Grand (1925) have shown that experimental lesions of the hypothalamus are sometimes followed by glycosuria and hyperglycemia. Beattie, Brow, and Long (1930) have described nuclei in the diencephalon which send direct fibers to those columns of gray matter in the spinal cord known to give rise to preganglionic sympathetic fibers. Houssay and Molinelli (1925) state that stimulation of the hypothalamus increases adrenalin secretion. Himwich and Keller (1930) found that direct stimulation of the hypothalamic region causes a rise in blood sugar. This morphological and physiological evidence clearly proves that centers above the medulla oblongata can and do play a part in controlling the mobilization of carbohydrates. The experiments reported in this paper indicate, however, that those higher centers are not necessary for reflex blood sugar rises.

SUMMARY

An effort was made to ascertain whether or not a bulbar control of carbohydrate mobilization exists, by comparing reflex rises in blood sugar induced before and after various parts of the central nervous system had been excluded. Cats were used exclusively.

1. To ascertain the reflex hyperglycemia of intact anesthetized animals 26 experiments were performed. The average reflex rise was 20 mgm. per cent in 10 cats under intravenous sodium barbital, 29 mgm. per cent in 6 animals under the same anesthetic injected intraperitoneally, and 22 mgm. per cent in 10 under chloralose.

2. In 10 thalamic animals studied under intravenous sodium barbital the average reflex rise was 26 mgm. per cent.

3. Reflex hyperglycemias were investigated in 26 decerebrate preparations. The average rise was 27 mgm. per cent in 10 cats under intravenous sodium barbital, 41 mgm. per cent in 6 animals under the same anesthetic injected intraperitoneally, and 20 mgm. per cent in 10 under chloralose.

4. Stimulation of an afferent nerve had only a very slight or no effect upon the blood sugar levels of the 15 decapitate animals which were studied.

5. In 10 experiments a diagonal section through the medulla oblongata from a point a millimeter or two rostral to the union of the cerebellum and brachium pontis dorsally to the posterior border of the pons ventrally did not abolish reflex hyperglycemia, for an average rise of 22 mgm. per cent was obtained in 6 animals under intravenous sodium barbital and a rise of 32 mgm. per cent in 4 animals under chloralose. But a section a few

millimeters caudal to that level abolished the phenomenon almost entirely in the 4 experiments performed and the animals reacted very similarly to the decapitate preparations.

It is concluded that there is in the floor of the fourth ventricle just posterior to the middle of the brachium pontis and very close to the vasomotor center a neural mechanism which is responsible for the reflex rises in blood sugar which result when an afferent nerve is stimulated in the anesthetized cat.

I wish to express my indebtedness to Dr. Philip Bard for suggesting this problem and for his unfailing helpfulness and encouragement.

BIBLIOGRAPHY

Bailey, P. and F. Bremer. 1921. Arch. Int. Med., xxviii, 773.
Bard, P. 1928. This Journal, lxxxiv, 490.
1929. Arch. Neurol. and Psychiat., xxii, 230.
Beattie, J., G. R. Brow and C. N. H. Long. 1930. Proc. Roy. Soc., B., cvi, 253.
Bernard, C. 1858. Leçons sur la physiologie et la pathologie du système nerveux.
Brugsch, T., K. Dresel and F. H. Lewy. 1920. Zeitschr. f. exp. Path. u. Therap., xxi, 2.
1921. Zeitschr. f. d. ges. exp. Med., xxv, 262.
Bulatao, E. and W. B. Cannon. 1925. This Journal, lxxii, 295.
Camus, J. and G. Roussy. 1922. Rev. neurol., xxxviii, 622.
Camus, J., J. J. Gournay and A. Le Grand. 1925. Presse méd., xxxiii, 249.
Cannon, W. B. and S. W. Britton. 1925. This Journal, lxxii, 283.
Cannon, W. B. and D. Rapport. 1921. This Journal, lviii, 338.
Carrasco-Formiguera, R. 1922. This Journal, lxi, 254.
Eckhard, C. 1869. Beitr. z. Anat. u. Physiol., iv, 4.
Elliott, T. R. 1912. Journ. Physiol., xliv, 374.
Folin, O. 1929. Journ. Biol. Chem., lxxxii, 83.
Griffith, F. R. 1923. This Journal, lxvi, 618.
Hiller, F. and A. Tannenbaum. 1929. Arch. Neurol. and Psychiat., xxii, 901.
Himwich, H. E. and A. D. Keller. 1930. This Journal, xciii, 658.
Houssay, B. A. and E. A. Molinelli. 1925. Compt. rend. Soc. biol., xciii, 1454.
Kahn, R. H. 1917. Pflüger's Arch., clxviii, 326.
Mellanby, J. 1920. Journ. Physiol., liii, 1.
Morita, S. 1915. Arch. f. exp. Path. u. Pharm., lxxviii, 188.
Olmsted, J. M. D. and H. S. Coulthard. 1927. This Journal, lxxxiii, 513.
Sachs, E. and M. E. MacDonald. 1925. Arch. Neurol. and Psychiat., xiii, 335.
Simnitzky, W. S., A. A. Wischnewsky and S. A. Satwornitzkaja. 1930. Pflüger's Arch., ccxxv, 648.

THE SECRETION OF PHENOL RED BY THE MAMMALIAN KIDNEY

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Eight years ago, Marshall and Vickers (1923) submitted evidence that phenol red (phenolsulphonephthalein) was secreted by the convoluted tubules of the dog's kidney as well as being eliminated by the glomeruli. Their conclusions were *1*, that an accumulation of phenol red in the cells of the convoluted tubules of the non-secreting kidney (lowered blood pressure) occurs after intravenous injection; *2*, that phenol red is present in dog's blood plasma to a large extent in some non-diffusible combination with the colloids; and *3*, that under certain conditions after intravenous injection a sufficient concentration of the filterable phenol red is not present in the arterial blood to account for the quantity eliminated in the urine even if filtration of all the plasma is assumed to occur. This last conclusion was based on certain assumptions, but the latitude used in making these assumptions was such that it seemed certain that the excretion of phenol red could not be accounted for entirely by filtration.

The subsequent work of Marshall and Crane (1924) supported this conclusion, and Starling and Verney (1925) using the heart-lung-kidney preparation confirmed and accepted the results. Some investigators, however have felt unable to accept this interpretation, and have presented criticisms of the experimental work. It has, therefore, seemed advisable to extend these investigations on the excretion of phenol red in the light of the knowledge of kidney function which has accumulated in the interval. The new experiments reported in this paper have been planned so as to avoid the assumptions, however likely, necessary for drawing conclusions from the previous work. The results confirm our former conclusion that phenol red is in large part excreted by tubular secretion in the dog.

The first series of experiments were in design like the earlier ones of Marshall and Vickers, with certain important modifications. Unanesthetized female dogs were used in determining the concentrations of phenol red present in blood plasma in relation to the rate of its excretion in the urine. This allows one to calculate the amount of plasma cleared of either total or filterable phenol red per minute and to estimate the minimum blood flow through the kidney necessary for its excretion by filtration alone.

In order to minimize uncertainties in regard to the amount of phenol red present in the dead space of the kidney (tubules, pelvis and ureter) at the beginning of the experimental period, subcutaneous injections were made, and the periods of urine collection prolonged. Special experiments showed that after subcutaneous injection the maximum concentration of phenol red is present in the blood in about one-half hour. The collection of urine was, therefore, begun at this time when the urine of the dead space would be of a lower concentration than that eliminated during the first part of the experimental period.

In the previous experiments, plasma concentrations were around 4 or 5 mgm. per cent: in the present experiments, concentrations of one-tenth or one-twentieth of these values were used. The amounts of phenol red injected in experiments designed to detect its secretion by the tubule should be as small as can be accurately estimated in plasma. Marshall and Crane (1924) showed that with increasing plasma concentrations, the efficiency of the kidney in excreting this substance was reduced, presumably due to a relatively lessened tubular secretion. This has now been definitely proven in the case of the aglomerular kidney of fish, as is shown in the following experiment.

Five toadfish (330 to 390 grams), injected intramuscularly with 1.8 mgm. of phenol red, secreted 12 to 23 per cent of the amount injected in five hours. Three toadfish (300 to 450 grams), injected with 36.0 mgm., secreted only 1 to 2 per cent in five hours. Fish of approximately the same weight showed plasma concentrations of 0.6 mgm. per cent with the smaller dose, and of 19 mgm. per cent with the larger dose two hours after injection. In the case of the smaller dose, the urine may contain 250 times the concentration of phenol red as the plasma; while with the larger dose, a concentration ratio of only 14 has been found. Here, then, tubular secretion is only about 10 per cent as efficient with the larger as with the smaller dose.

The plasma clearance for creatinine after its ingestion, which has been suggested by Rehberg (1926) as a measure of the amount of glomerular filtrate, was determined on the same animals as were used for the phenol red experiments. In some experiments creatinine alone was used while in other experiments phenol red and creatinine were administered together.

The analyses of phenol red and creatinine in plasma and urine were made by means of a Bürker colorimeter. Phenol red was estimated in plasma directly by the addition of alkali, with a sample of plasma taken from the same dog before the experiment used to compensate for the plasma color. This method has been tested frequently and found accurate. Creatinine was determined by Jaffe's color reaction. By compensating for the phenol red color, creatinine could be easily determined in the presence of phenol red.

Two dogs were used for these experiments which were performed at various intervals from October to April. The procedure was as follows:

Blood was drawn from the saphenous vein, phenol red solution (about 6 mgm. per kilo) injected subcutaneously, and usually 250–300 cc. of water with or without 6 grams of creatinine given by stomach tube. The bladder was emptied by catheter at the end of one-half, one and one-half and two and one-half hours. Blood was drawn from the saphenous vein or femoral artery either at the beginning and end of each hourly period of urine collection or at the mid-point of the period. Either heparin or oxalate was used to prevent coagulation. The animals lay quietly on a blanket on the floor, except when blood or urine was being collected.

A brief summary of the results on one dog is given in table 1.

TABLE 1

Police dog, 19.0 kilo

DATE	URINE	PHENOL RED		PLASMA CLEARED PER MINUTE	CREATININE		PLASMA CLEARED PER MINUTE
		Plasma	Urine		Plasma	Urine	
	cc. per minute	*mgm. per cent*	*mgm. per minute*	*cc.*	*mgm. per cent*	*mgm. per minute*	*cc.*
10–28	0.162	0.43	0.62	144			
3–19	1.43	0.43	0.66	154			
	0.58	0.21	0.29	139			
3–24	1.65	0.38	0.56	147			
	0.50	0.25	0.32	128			
3–27	3.17				17.0	15.7	92
	1.08				13.3	11.4	85
4–21	2.62	0.46	0.53	115	19.4	13.7	70
	0.95	0.23	0.34	148	15.4	8.6	56
4–28	2.93	0.54	0.73	135	20.0	15.7	79
	1.09	0.21	0.31	148	13.0	13.0	100

Ultrafiltration experiments on blood plasma taken from this dog showed that only 25 per cent of the total phenol red was in the filterable form. Hematocrit readings gave an average of about 50 per cent corpuscles. If we take a plasma clearance of 150 cc. per minute and multiply by four we have the amount of plasma, 600 cc., necessary to contain the filterable phenol red to account for the amount excreted by the kidney per minute. This means that the blood flow through the two kidneys must be 1200 cc. per minute, even if we assume that all the plasma is filtered at the glomeruli.[1] It is, of course, obvious that all the plasma cannot be filtered and it is quite probable that the filtered fraction is not more than 50 per cent. On this basis, we need a blood flow of 2400 cc. per minute. This last is almost as much as the total cardiac output per minute of a dog of this size (Marshall, 1926), while the figure for blood flow (1200 cc.)

[1] Phenol red is contained in blood only in the plasma and not in the red corpuscles.

on the assumption of filtration of all the plasma is nearly half of the total heart output. It is obvious that the blood flow through the kidneys is not of this magnitude, and hence that glomerular filtration alone cannot account for the phenol red eliminated. Recently Rhoades, Van Slyke, Hiller and Alving (1931) have reported that the rate of blood flow through one kidney of an unanesthetized dog of this size is in the neighborhood of 200-250 cc. per minute.

In table 1 it is evident that the figures for plasma clearance of creatinine are consistently lower and much more variable than those for phenol red. If the figures for phenol red clearance are multiplied by 4 to get the clearance of free phenol red, the difference is much more pronounced. On Rehberg's assumption that the creatinine clearance is a measure of glomerular filtrate, we can calculate that only 9.5 to 17.0 per cent of the total red is excreted in the glomerular filtrate. If a part of the creatinine is secreted by the tubules the amount of phenol red in the glomerular filtrate will be correspondingly less.

The data obtained on the second dog, "Beauty," 15 to 16 kilos, are essentially the same. Determinations of plasma clearance in cubic centimeters per minute for phenol red from November to February were consecutively: 144, 146, *92*, *99*, 122, 126, 136, 136, 145, 147, *87*, *93*. The first pair of figures in italics represent the result when ten times the usual dose was given (plasma = about 4 mgm. per cent), while the second pair of italicized figures were obtained for injection of twenty times the usual dose (plamsa = about 7 mgm. per cent). The creatinine clearances were 65, 90, 76, 60. It is here evident that the kidney is less efficient in excreting phenol red from a high than from a low plasma concentration.

In the case of the subcutaneous injections of phenol red used in these experiments it is difficult to imagine secretion of this substance to occur from a store laid up in the renal cells at the time of injection. The other alternative, which can explain the high clearance values found, is that both free and combined phenol red are excreted in an extremely efficient manner by the kidney (Marshall and Vickers, 1923).

It has been possible to test this idea by a comparison of the phenol red concentrations in arterial and renal vein blood. Since our previous experiments were done on dogs anesthetized with paraldehyde and seemed to show a high plasma clearance for the particular dose used, a few experiments on dogs under this anesthetic were done in the same manner as those on the unanesthetized dogs recorded above. The plasma clearance values obtained were of the same order of magnitude as those on unanesthetized dogs for both phenol red and creatinine.

A second series of experiments was therefore carried out on dogs anesthetized with paraldehyde to compare the phenol red content of arterial and renal vein blood. The renal vein blood was obtained by inserting a

needle into the left spermatic or ovarian vein and pushing it into the renal. This procedure involved no dissection or handling of the kidney or renal vessels. In two instances the valve at the entrance of the spermatic into the renal vein was found to be lacking or incompetent and blood was drawn directly from the spermatic vein. In all cases the spermatic or ovarian vein was occluded pheripherally to the renal before drawing blood. In a few instances the renal vein was exposed and blood drawn directly from it. Phenol red (6 mgm. per kilo) was given subcutaneously one-half to one hour and a half before drawing blood samples.

The following protocol illustrates the details of an experiment.

Dog, male, 14.2 kilos. At 9:15, given 27 cc. paraldehyde and 200 cc. water by stomach tube. 10:30 to 10:50, tracheal cannula inserted, and left carotid artery isolated. At 10:54, 10 cc. of blood drawn from carotid: at 10:55, 15 cc. (90 mgm.) phenol red injected subcutaneously. From 11:15 to 11:23, exposed left spermatic vein by lumbar incision (extraperitoneally). At 11:46, 6 cc. of blood drawn from renal vein by inserting needle through spermatic vein. At 11:47, 10 cc. blood drawn from carotid artery. About 5 mm. of renal vein exposed half way between kidney and vena cava. At 12:04, 10 cc. blood from renal vein. At 12:04:30, 10 cc. of blood from carotid artery. Dog bled 300 cc. from carotid.

TABLE 2

Phenol red content of arterial and renal vein bloods

EXPERIMENT NUMBER	PHENOL RED		PER CENT REMOVED	REMARKS	PER CENT PHENOL RED IN ULTRAFILTRATE
	Arterial	Renal vein			
	mgm. per cent	*mgm. per cent*			
1	0.50	0.17	66	Spermatic, valve incompetent, injection of sodium sulfate	22
2	0.48	0.32	34	Renal vein	20
3	0.40	0.15	63	Spermatic	25
	0.37	0.185	50	Renal vein	
4	0.43	0.15	65	Ovarian	24
	0.37	0.20	46	Ovarian, trauma	
5	0.48	0.15	69	Spermatic, valve incompetent	
6	0.45	0.20	55	Renal vein	

First pair of samples, arterial plasma contained 0.40 mgm. per cent of phenol red, and renal vein plasma (via spermatic) contained 0.15 mgm. per cent (62.5 per cent removed by kidney).

Second pair of samples, arterial plasma contained 0.37 mgm. per cent of phenol red, and renal vein plasma, 0.18 mgm. per cent (49 per cent removed by kidney).

An ultrafiltrate of serum contained 0.09 mgm. per cent phenol red as opposed to a total content of serum of 0.37 mgm. per cent (25 per cent free).

Table 2 gives a comparison of the phenol red content of arterial and renal vein plasma from six dogs. In one animal, the left ureter was cannulated,

an injection of sodium sulfate given intravenously, and urine collected. The plasma clearance for phenol red before exposing the spermatic vein was 45 cc. per minute (one kidney). A second determination collecting urine for a few minutes before, during and after drawing renal vein blood was 53 cc. per minute.

The renal vein plasma has varied from 31 to 66 per cent of the arterial plasma in its phenol red content. In the five cases where blood was obtained via the spermatic or ovarian veins, its plasma content in phenol red has been 31, 34, 37, 35 and 54 per cent of that of the arterial blood plasma. The high value (54) was obtained in a second sample of blood drawn via the ovarian vein, where considerable difficulty was experienced in drawing blood. The most perfect experiments technically therefore give a phenol red content of renal vein blood of 31 to 37 per cent of that of arterial blood (63 to 69 removed by the kidney).[2] Ultrafiltration of blood serum obtained at the end of these experiments showed that from 20 to 25 per cent of the phenol red was in the filterable form.[3] Thus, we see that the kidney can remove 63 to 69 per cent of the total phenol red present in arterial blood plasma under conditions where only 20 to 25 per cent of the total phenol red of arterial plasma is filterable. It is obvious that filtration and reabsorption as the sole processes taking place in the mammalian kidney cannot explain this result. Indeed, it appears that less than 20 per cent of the phenol red excreted by the kidney can be accounted for by glomerular filtration (assuming 50 per cent of the plasma to be filtered).

The outcome, then, of these two series of experiments is to confirm the conclusion drawn from our previous work, that it is necessary to assume the participation of some other factor than glomerular filtration and tubular reabsorption to explain the excretion of phenol red. Richards (Richards and Barnwell, 1927; Richards, 1929) has criticized our earlier experiments on three grounds; namely, *1*, that the fraction of phenol red which can be ultrafiltered through a collodion membrane is not the fraction which is free to pass through the glomerular membrane; *2*, that our assumption of a rate of blood flow through the kidneys of 5 cc. per gram per minute is too low, and *3*, that adequate account was not taken of the amount of phenol red actually present within the lumina of the tubules in concentrated form at the very beginning of our experimental periods.

[2] The lymph flow from the kidney will introduce an unknown error into the comparison of arterial and renal vein blood. However, this error is probably very small and in any case tends to increase the difference in phenol red content between the two bloods.

[3] Grollman (1925) found 25 per cent in the ultrafiltrate from dog's plasma. Our values obtained in these experiments are essentially the same as those of Grollman. The higher values (25 to 40 per cent) obtained in the earlier work of Marshall and Vickers (1923) are explained by the fact that the plasma proteins were frequently diluted due to the intravenous injection of large quantities of salt solutions.

In regard to the first point, it is quite clear that if the glomerulus allows more phenol red to pass than the fraction which is present in the plasma in filterable form either the glomerular filtrate normally contains protein or the process by which phenol red is excreted at the glomerulus is not entirely filtration. All of the evidence available indicates that glomerular fluid is normally free of protein (Marshall and Vickers, 1923; Ekehorn, 1930). Proof of the combination of phenol red with the plasma proteins is not dependent on the use of a collodion membrane. Grollman (1925, 1926) has given a qualitative optical proof of the absorption of phenol red on plasma proteins and has found quantitative agreement of the results obtained by ultrafiltration and by dialysis. The equilibrium between the free and combined phenol red in blood plasma is independent of any particular membrane or the use of any membrane at all. Therefore, if some of the combined phenol red is eliminated at the glomerulus, the process cannot entirely be one of filtration. Richards has apparently recently accepted the above viewpoint since his experimental finding in the frog (Richards and Walker, 1930) that "The values of the concentrations of phenol red and indigo carmine in glomerular urine collected during the renal elimination of these dyes bear the same relation to their concentrations in the blood plasma as is obtained when plasma is filtered through collodion" is used as evidence that glomerular excretion is purely a process of filtration.

In the matter of blood flow through the kidney Richards says, "Doubtless we shall never know what the actual figures of maximal rate of blood flow through the kidney under normal conditions are, but I am strongly of the opinion that in Marshall's experiments they must have been higher than he believed, and his conclusions would, I think, have been modified had the figure 8 been used instead of 5 for maximal blood flow." Calculation from the figures given in the two protocols in our former paper indicate that our conclusions would not have been modified by using the figure 8 instead of 5 unless one is willing to assume that 62 and 75 per cent of the plasma can be ultrafiltered at the glomerulus. Since the present experiments completely answer this point, no further comment is necessary.

The third point in regard to the phenol red present in the dead space of the kidney at the beginning of the experimental periods was discussed in our former paper, and is completely covered by the present experiments.

Cushny (1926) and Ekehorn (1930) have also suggested that the glomerulus may split the combined phenol red from the plasma proteins and eliminate it. As shown above, however, this amounts to admitting "secretion" of the substance at the glomerulus.

Ekehorn further says in regard to combined phenol red "In any case the compound must be split up also if the tubular cells secrete its dye-component; and this splitting must occur in the blood of the capillaries because

the dye component cannot permeate the capillary wall . . . In other words, it is no more possible to localize the excretion of nondiffusible dyes to secreting renal tubules than it is to localize it to filtering glomeruli. . . ." The experiments on the comparison of arterial and renal vein blood prove that a large proportion of the combined phenol red in plasma is in fact removed by the kidney, and we see no way in which this can be accomplished by filtration alone. The combined phenol red need not permeate the capillary wall. With the passage of the free phenol red into the tubule cells, the equilibrium is disturbed and more phenol red is liberated from the combined form.

Rehberg (1926) and Ekehorn (1930) argue from *a priori* considerations that secretion by the kidney must be a less efficient process than filtration and that it must require a greater rate of renal blood flow. However, the facts show that some process in the kidney is more efficient in requiring less blood flow for the elimination of phenol red than filtration would; about 70 per cent of the total phenol red in plasma can be removed by the kidney, where the same rate of blood flow could remove less than 20 per cent by filtration alone.

MacKay and Oliver (1930) while admitting tubular secretion of neutral red state that phenol red is eliminated entirely by the glomerulus in the mammalian kidney. They find for phenol red a direct relationship between the plasma concentration and rate of excretion which is opposite to the results of Marshall and Crane (1924). They attempt to explain this discrepancy by suggesting that Marshall and Crane used too small urine volumes, too short times of collection, and drew blood samples at the wrong time. Since in our experiments urea was injected together with the phenol red, it is evident that the above factors should have affected the urea figures in the same way as those for phenol red. But their results for urea confirm ours. A much more likely explanation of the discrepancy of our results and those of MacKay and Oliver is to be found in the dosage of phenol red employed, for they have worked with plasma concentrations of 10 to 150 mgm. per cent, values of a much higher order of magnitude than those reported in our work and we have shown that the efficiency of the kidney in excreting phenol red decreases with increasing plasma concentrations.

It is interesting to note that if we assume 70 per cent removal of phenol red by the kidney for our unanesthetized dogs, the rate of blood flow through the kidney calculated from the clearance figures of table 1, works out to be 165 to 220 cc. per minute (one kidney). This agrees well with the figures reported for the unanesthetized dog by Rhoades, Van Slyke, Hiller and Alving (1931).

The data presented in the present paper point unmistakably to the conclusion that over 80 per cent of the phenol red excreted by the mammalian

kidney is excreted by some other process than that of glomerular filtration. The specialized epithelium of the convoluted tubule as opposed to the thin epithelium investing the glomerular tuft, the accumulation of phenol red in the cells of the convoluted tubules of the non-secreting dog's kidney, and the fact that phenol red is secreted by the tubule of the aglomerular fish kidney all point to the elimination of phenol red by the tubule. In the case of the toadfish kidney, it is now certain that secretion of phenol red is accomplished by the analogue of the proximal convoluted segment of the mammal (Bieter, 1931; Grafflin, 1931): it is reasonable to suppose that this same proximal segment secretes phenol red in the mammal. As to its secretion by other segments of the mammalian tubule, however, nothing is known.

No attempt will be made in the present paper to discuss the secretion of phenol red by other classes of vertebrates or to consider what the relatively large proportion of phenol red eliminated by tubular secretion as opposed to glomerular filtration means in regard to the excretion of the normal urinary constituents. It is important, however, to point out that filtration-reabsorption and tubular secretion are not incompatible as some recent writers have assumed, but that both processes operate in the same kidney. What is most needed at present is a knowledge of the relative importance of these two processes in the elimination of various substances by the kidneys of different classes of vertebrates. This has been recently discussed in another place (Marshall, 1931).

SUMMARY AND CONCLUSIONS

The mammalian kidney is much more efficient in excreting phenol red than creatinine. Taking account of the fact that only a small amount of phenol red in dog's plasma is filterable, it is shown that the blood flow through the kidney necessary for the excretion of this substance by filtration alone is of an impossible order of magnitude. A comparison of the phenol red content of arterial and renal vein blood plasma indicates that 70 per cent of the phenol red may be removed by the kidney, while only 25 per cent is filterable.

Filtration alone therefore will not account for the amount of phenol red eliminated. The other process in its elimination is tubular secretion, which accounts for over 80 per cent of the total amount excreted. This confirms the work of Marshall and Vickers, and answers criticisms which have been made of this earlier work.

BIBLIOGRAPHY

BIETER, R. N. 1931. This Journal, xcvii, 66.

CUSHNY, A. R. 1926. The secretion of the urine. Longmans, Green & Co., London.

EKEHORN, G. 1930. On the principles of renal function. Stockholm. (Acta med. Skand., Supp. xxxvi).

GRAFFLIN, A. L. 1931. Bull. Johns Hopkins Hosp., xlviii, 269.
GROLLMAN, A. 1925. Journ. Biol. Chem., lxiv, 141.
1926. Journ. Gen. Physiol., ix, 813.
MACKAY, E. AND J. OLIVER. 1930. Journ. Exper. Med., li, 161.
MARSHALL, E. K., JR. AND J. L. VICKERS. 1923. Bull. Johns Hopkins Hosp., xxxiv, 1.
MARSHALL, E. K., JR. AND M. CRANE. 1924. This Journal, lxx, 465.
MARSHALL, E. K., JR. 1931. The kidney in health and disease. Univ. of Minn. Symposium, July, 1930. P. B. Hoeber & Co.
REHBERG, P. B. 1926. Biochem. Journ., xx, 453.
RHOADES, C. P., D. D. VAN SLYKE, A. HILLER AND A. ALVING. 1931. Proc. Soc. Exp. Biol. Med., xxviii, 776.
RICHARDS, A. N. AND J. B. BARNWELL. 1927. Proc. Roy. Soc. B., cii, 72.
RICHARDS, A. N. 1929. Methods and results of direct investigations on the function of the kidney. Williams and Wilkins, Baltimore.
RICHARDS, A. N. AND A. M. WALKER. 1930. Journ. Biol. Chem., lxxxvii, 479.
STARLING, E. H. AND E. B. VERNEY. 1925. Proc. Roy. Soc. B., xcvii, 321.

STUDIES IN THE MOTOR ACTIVITY OF THE LARGE INTESTINE

II. The Influence of Balloon Technique upon Colon Motility

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The accuracy of records of gastro-intestinal motor activity taken by means of balloons distended within the lumen has often been questioned. Welch and Plant, 1926, observed that tone and activity in the proximal colon of unanesthetized dogs were greater during the first portion of the tracing than later, and ascribed the augmentation to the insertion of the recording balloon through the cecostomy, and its inflation.

As early as 1900 Bayliss and Starling had confirmed their "law of the intestine" on the colon of anesthetized dogs, while Langley and Magnus in 1905 found occasionally a reversal of the law in the distal colon of the rabbit. Elliott and Barclay-Smith reported in 1904 that distention of a balloon in the distal colon of the cat induced colon peristalsis.

In 1926 Currie and Henderson noted that increasing the pressure within the guinea pig's proximal colon produced longitudinal contractions of sufficient force to expel the contents, with low peristaltic contractions, presumably involving the circular muscle fibres. Similarly Fleisch, in 1928, observed a shortening of the guinea pig's colon as a response to mechanical stimulation.

Weitz and Vollers, in 1927, inflated balloons in the rectum in man and observed depression of tone and activity in their recording balloon, inserted by rectum into the distal colon. Peiper, however, in 1928, working on the distal colon of children, was able to detect no alteration of activity in a two-balloon record on over-inflating one of the balloons.

In 1926 Pearcy and Van Liere, working on the effect of distentions of the gastro-intestinal tract in unanesthetized dogs and cats, suggested that adequate distention of any portion of the tract inhibits all other portions except the ileo-cecal sphincter. Their balloons were distended under pressures as high as 50 mm. Hg.

Early in our study of the activity of the colon in unanesthetized dogs, we observed irregularities in the first portion of the tracings similar to those

which are reported by Welch and Plant, and discarded this portion of the records in the presentation of the normal. We were using at that time triple tandem balloons inserted into the proximal colon by way of cecostomies, under conditions which were quite comparable to those of Welch and Plant. On introducing the use of two triple balloon systems, one inserted through the cecostomy, and the other through the anus (Templeton and Lawson, 1931) we considered it necessary to determine the effect which the insertion of each set might have on the activity recorded by the other, and also the influence which moderate over-inflation of each of the recording balloons, with pressures comparable to those obtaining at the height of tone changes against a recording balloon, might have on its own and other segments of the colon.

For the study of the effect of insertion of one of the balloon systems, tracings were started with either the proximal or the distal set in place and inflated under the pressures used for recording. The volume of balloon, lead-tube, and manometer was such that a 10 cm. inflation of the water manometer with the balloon system clamped off, furnished a pressure of about 2 to 3 cm. of water against the uncontracted gut wall, when the balloon system was unclamped. When constant activity was attained on this record, usually within one to two hours after the insertion of the balloons, the second set was inserted, and at times inflated immediately for recording the activity of the segment itself, at times left uninflated so as to record the effect of the insertion alone on the other set of balloons.

With all six balloons inflated to recording pressures, as previously described, individual balloons were moderately over-inflated with an additional 10 cm. of water directly against the gut wall, so that pressures were exerted aginst the wall of the colon from three to six times as great as were used in obtaining the normal records. Similarly the effect on adjacent segments of deflating one of the recording balloons was studied.

In order to localize more definitely the effects observed on inserting the distal set of balloons, multiple stimulating electrodes were inserted through the anus along with the distal set of balloons, so that different points in the distal colon could be selectively stimulated without moving the electrodes, so as to avoid introducing possible error by mechanical irritation. No attempt was made to determine the strength of current required to stimulate in this manner. Moderate tetanizing currents were employed, and the strength increased gradually until the dog showed signs of discomfort. Frequently movements of the tail, perineal muscles, and sometimes even the hind legs were observed.

When the distal set of balloons was inserted while a tracing was being made from the proximal colon, it was observed that almost immediate depression of tone and activity in the proximal colon ensued, and lasted from 5 to 30 minutes. This has been an almost invariable observation on 15

dogs. If the distal set of balloons was inserted during a period of inactivity in the proximal colon, the proximal tracing usually showed a brief single contraction simultaneously on all the balloons, with immediate return to inactivity. This effect was obscured if the proximal colon was active at the time of the insertion. If, while a tracing was being taken from the entire colon, with both sets of balloons inserted and inflated, a rubber tube was inserted through the anus into the distal colon, the effect on both proximal and distal colon was observed (fig. 1). During the period of proximal colon depression the distal colon is greatly augmented in tone, and high type 1 contractions appear, which are gradually supplanted by type 2 contractions as tone falls. This augmented activity,

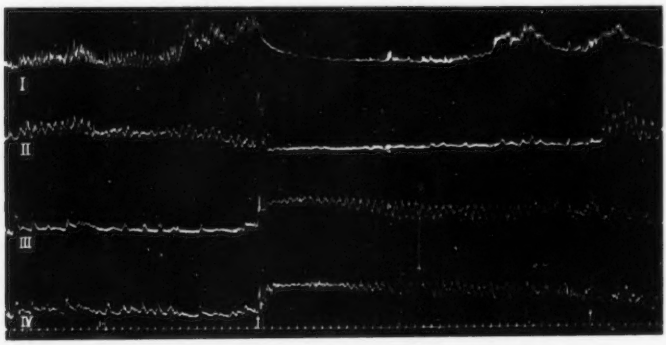

Fig. 1. Four-balloon tracing of entire colon. Balloons I and II are in the proximal colon, separated by a distance of 9 cm.; balloons III and IV a similar distance apart in the distal colon, balloon I at level of ileocecal sphincter, balloon IV just within the internal anal sphincter. Time interval, 1 minute. At the point indicated by an arrow a stiff rubber tube 1 cm. in diameter was inserted through the anus into the distal colon. Balloon I recovers tone and activity within about 30 minutes, balloon II returns in about 45 minutes.

especially the high type 1 contractions, is more marked on the balloon which lies nearest the sphincter area. When the proximal colon had recovered, and activity had become constant in both segments, shifting the position of the distal balloons in either direction had the same effect as the insertion of the balloons. On the other hand, we have made continuous 6-balloon tracings from the dog's colon for as long as 22 hours, without marked alteration of activity after the first one or two hours, so long as the position of the balloons was unaltered. In these long tracings we have observed a tendency toward cyclical changes in activity, but no progressive diminution.

The insertion of the proximal set while a tracing was being taken from

the distal colon has not produced any constant effect on distal colon activity. In one dog shifting the position of the proximal set was usually followed by diminution in activity in the distal balloons. The insertion or removal of the proximal set seemed, in this animal, to be attended with more discomfort than in other dogs.

Inflation of individual balloons in the distal colon with 10 cm. of water pressure in addition to the recording pressure, has produced little alteration in the activity of any segment. The slightly augmented amplitude

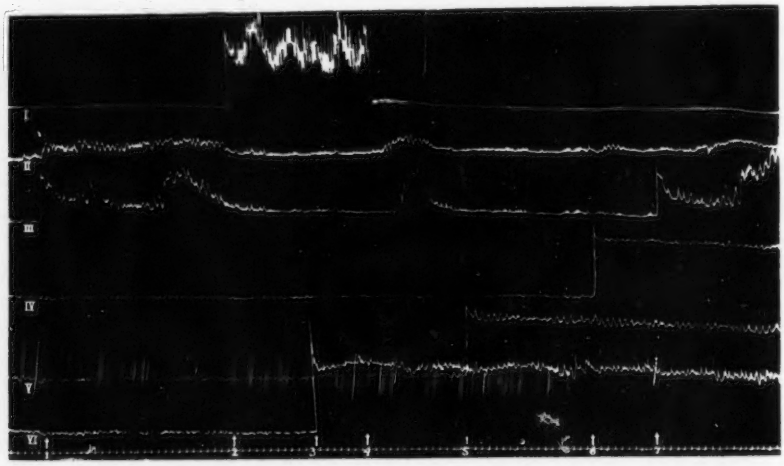

Fig. 2. Six-balloon tracing of entire colon. Balloon I at ileocecal sphincter, balloon VI just within internal anal sphincter. Interval between adjacent balloons in either set 5 cm. Time interval, 1 minute. Method of inflation furnishes about 2 cm. of water pressure against the wall of the uncontracted gut. The points indicated by arrows and numbers *1* to *7* are inflations of individual balloons with approximately equal additional pressures, except that 4 is deflation of balloon I, following over-inflation. The over-inflation never exceeded 10 cm. of water. The depression of II and III during this over-inflation of I could not be repeated. Balloon III was over-inflated at *1* and *7* without deflation, and without maintaining high tone. Balloon II was kept throughout unaltered as a control.

of contractions in the over-inflated balloon may be due either to stimulation of the segment, or to the increased volume of air displaced by contractions of the same amplitude (fig. 2). We have not recorded in the distal colon a rise in tone following the over-inflation. In the proximal colon, on the other hand, increasing the pressure in one of the balloons by 10 cm. of water has usually been followed by increased amplitude of contractions, a rise in tone, frequently preceded by a fall; while segments above the distention record also slightly increased tone and activity (fig. 2).

Little effect has been observed on the distal colon, on thus augmenting proximal colon activity.

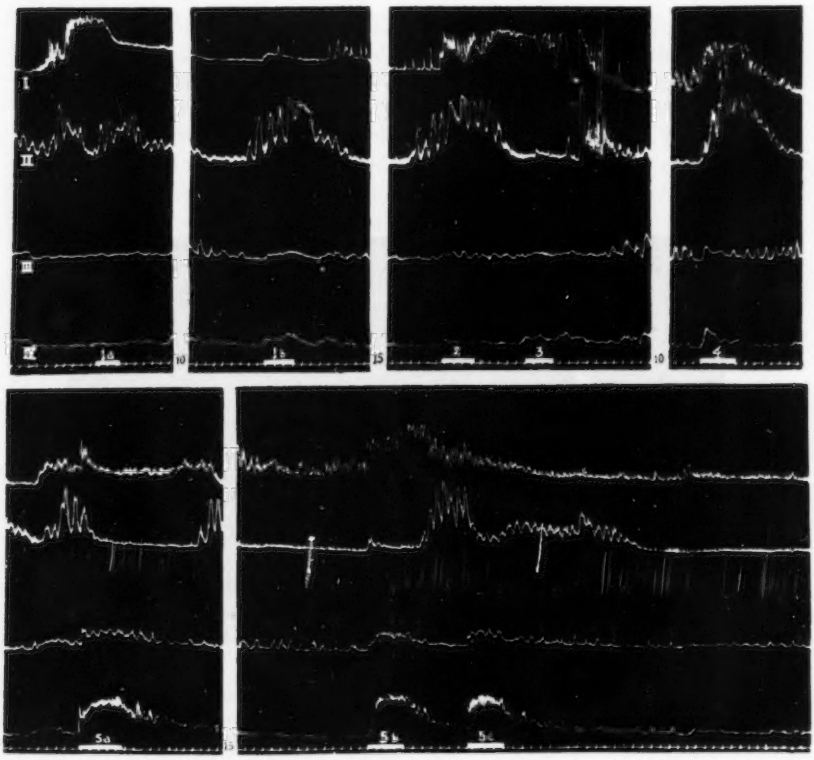

Fig. 3. Four-balloon tracing of entire colon. Balloons I and II are in the proximal colon, separated by a distance of 9 cm., balloon I at level of ileocecal sphincter. Balloons III and IV are similarly spaced in the distal colon, balloon IV just within the internal anal sphincter. Time interval, 1 minute. The points indicated are equal tetanizing stimuli applied to different levels in the distal colon. *1* is stimulation just above the internal anal sphincter. *2* is same stimulus applied 5 cm. above internal anal sphincter, during period of proximal colon activity. *3* is same stimulus applied 10 cm. above the internal anal sphincter. At *4* the same stimulus was given at a point 15 cm. above the internal anal sphincter. *5 a*, *b*, and *c* are stimulations of the anal side of the internal anal sphincter with the same stimulus. During the stimulus *5 b* the effect in the distal colon is typical, but there is no evidence of depression of balloon I.

Electrical stimulation of the distal colon at different levels produced essentially negative results with current strengths which the dog would

tolerate, except when the region near the internal anal sphincter was stimulated, (fig. 3 and fig. 4). When this region was stimulated, the effect obtained was the same in both proximal and distal colon as when the distal set of balloons was inserted. The proximal colon was depressed, and the distal colon augmented in both tone and activity. The increased tone and the high type 1 contractions were always most marked in the balloon nearest the sphincter (fig. 4). Proximal colon depression was usually least complete on the balloon nearest the cecum (fig. 3) and this balloon was the first to recover from depression under either condition (fig. 1).

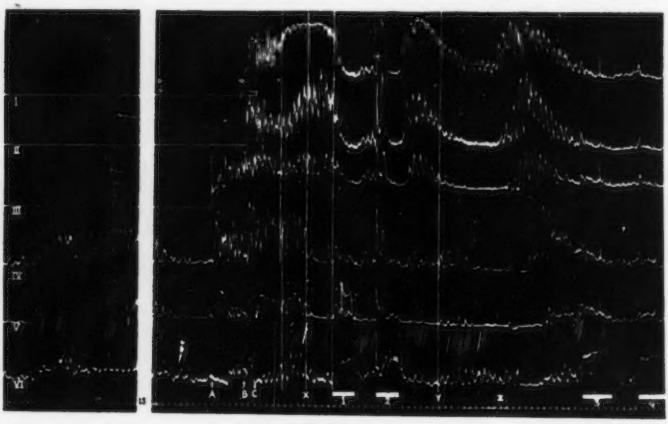

Fig. 4. Six-balloon tracing of entire colon. Balloon I at ileocecal junction, balloon VI just within internal anal sphincter. Interval between adjacent balloons in either set 5 cm. Time interval, 1 minute. The tracing was begun with proximal balloons inserted but uninflated. At *A*, *B*, and *C* the proximal balloons were inflated under recording pressures as indicated. *1*, *2*, *3*, and *4* are tetanizing currents applied to the internal anal sphincter during different phases of proximal colon activity. The letters *X*, *Y*, and *Z* refer to spontaneous alterations of colon motility.

The conditions under which these data have been gathered are hardly comparable to those under which other work of similar nature has been done. We have employed distentions which lie within the range used by us in our balloon study of the normal activity, while other workers have undoubtedly used distending pressures which were more effective stimuli. Even the moderate pressures which we have used have furnished confirmation of the observations of Bayliss and Starling and Welch and Plant, in the dog's proximal colon. Our data on the distal colon, on the other hand, agree with the observations of Peiper. We have not attempted to check the data of Pearcy and Van Liere, using pressures sufficient to

stimulate the distended segment. With our moderate distentions we do not duplicate the findings of Weitz and Vollers.

The concentration of activity to the region of the sphincter when the sphincter is stimulated, might suggest that activity appearing on other balloons of the distal set is referred from the activated sphincter. The alternate explanation is that the entire distal colon reacts to adequate stimuli in the same manner as the sphincter area.

SUMMARY

1. Moderate distentions of the proximal colon of the dog such as we have used in obtaining balloon records are followed by a temporary augmentation of proximal colon tone and activity.

2. Similar distentions of the distal colon do not alter the activity of any part of the colon, except for a possible slight local augmentation of activity without tone change.

3. Mechanical irritation of the anal sphincter area increases the tone and activity of that area, and possibly of adjacent segments, and depresses the tone and activity of the proximal colon.

4. Electrical stimulation of the anal sphincter region has the same local effect, and the same depressing effect on the proximal colon, as mechanical irritation of the region.

5. Similar electrical stimulation of other areas in the distal colon are less effective both locally and on the proximal colon.

The writers are indebted to Dr. A. J. Carlson, under whose direction this work was conducted.

BIBLIOGRAPHY

Bayliss and Starling. 1900. Journ. Physiol., xxvi, 107.
Currie and Henderson. 1926. This Journal, lxxviii, 287.
Elliott and Barclay-Smith. 1904. Journ. Physiol., xxxi, 272.
Fleisch. 1928. Pflüger's Arch., ccxx, 512.
Langley and Magnus. 1905. Journ. Physiol., xxxiii, 34.
Pearcy and van Liere. 1926. This Journal, lxxviii, 64.
Peiper. 1928. Jahrb. f. Kinderheilk., cxx, 312.
Templeton and Lawson. 1931. This Journal, xcvi, 667.
Weitz and Vollers. 1927. Arch. f. Verdauungskrankh., xli, 317.
Welch and Plant. 1926. Amer. Journ. Med. Sci., clxxii, 261.

STUDIES ON THE PHYSIOLOGY OF SECRETIN

IV. The Effect on the Secretion of Bile

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Bayliss and Starling (1902) reported intravenous injections of secretin to augment the flow of bile. Many writers since then have confirmed their findings. Whipple (1922) refers to secretin among other substances reported to cause minor fluctuations in the flow of bile. He states, "In conclusion we may repeat that the only active cholagogues are bile or bile salts." Mellanby (1928a), Lueth and Kloster (1928), and Still (1930) found that intravenous injection of very pure secretin preparations augment the flow of bile. On the other hand Takacs (1928) reported that secretin prepared by him had no effect on the rate of the bile flow.

Mellanby (1928b) investigated the mechanism by which secretin augmented the flow of bile. He was attracted by the long latent period of the liver response as compared with the pancreas and carried out many experiments from which he concluded: 1, that secretin, per se, had no effect upon the liver, and 2, metabolites from the active pancreas were carried via the portal blood to the liver where they stimulated the liver to produce more bile.

On numerous occasions we have observed that the continuous injection of sufficient purified secretin to maintain a fairly rapid pancreatic secretion was accompanied by a marked augmentation in the flow of bile. Should secretin be the agent causing the effect it seemed probable that during digestion sufficient secretin would be absorbed to cause a similar condition, and therefore secretin might have a more important rôle in the physiology of the liver than has been heretofore supposed.

In this paper we wish to present the results of our experiments which were planned to determine: 1, does secretin, per se, act as a cholagogue? and 2, by what mechanism do secretin preparations stimulate the liver?

Experimental. The secretin used in our experiments has been, in the main, from a large single sample (no. 372) prepared by the method described by one of us (E. U. S. 1930). It was vasodilatin free and of such activity that 1 mgm. or less caused a maximal response of the pancreas. It contained no choleocystokinin and had no effect on the blood sugar of dogs. All results were confirmed by using our preparations 401

and 413, which represent our most potent products. This procedure was followed in order to economize as much as possible on our most potent products, which were available in rather small amounts.

All comparative experiments have been done on the same dog (barbital). Solutions to be compared were injected only after the secretory rate of the pancreas and liver had returned to the spontaneous rate of the animal.

Effect of reagents on secretin. It has been observed that if unsterilized secretin solutions are permitted to stand at room temperature they rapidly lose activity. If they are heated with dilute acid or alkali, treated with proteolytic enzymes or heated for longer periods in the dry form the activity is destroyed. If one, by any of these means, destroys part of the pancreatic activity, an approximate reduction will be found on measuring the cholagogue activity.

TABLE 1

The pancreatic juice and bile collected following the intravenous injection of similar amounts of secretin activity in the form of crude or purified preparations

AMOUNT INJECTED	INCREASE PANCREATIC JUICE	INCREASE BILE	RATIO, PJ/BILE
mgm.	*cc.*	*cc.*	
35	4.8	0.7	6.86
8	5.1	0.9	5.66
2	5.0	0.7	7.14
1	4.3	0.6	7.16

Potency of various preparations. Several secretin preparations were selected at different stages of purification and assayed for pancreatic activity. Then such quantity of each preparation was injected as to represent the same amount of pancreatic stimulant. Obviously such an experiment was performed on the same dog and the secretory rate of the pancreas and liver was basal before an injection was made. The data from one such experiment are found in table 1.

The effects of the application of dilute HCl to the duodenum and the intravenous injection of secretin on the quantity of pancreatic juice and bile secreted were studied and compared. Since repeated applications of acid to the duodenum give rapidly diminishing responses, the comparisons have not been as satisfactory as in other types of experiments. However, we can say that in a given dog, when the pancreatic stimulation by HCl or secretin are parallel, the increase in the bile secreted will also be approximately parallel.

The work of Takacs suggests that the cholagogue effect of secretin preparations is due to some substance other than secretin. We have not been able to confirm his findings. Although secretin and the hypothetical

cholagogue may exist, it does not seem reasonable that they would always appear in the same concentration together, nor should the same reagents destroy them at approximately the same rate. It is also very difficult to conceive of the two substances being absorbed from the intestine at the same rate. Our data, we feel, justify one to conclude that the cholagogue and secretagogue effect of secretin is caused by one and the same substance.

Effect of secretin on the secretory pressure of the liver. It is a well known fact that the liver will secrete against a considerable pressure. If one clamps the cystic duct and connects the common bile duct to a bromoform manometer, the bile pressure will gradually rise to a maximum of 70 to 140 mm. bromoform pressure. After a time, if the pressure is maintained, resorption will begin and the pressure will gradually become diminished. If one injects 0.5–2 grams of Na cholate or better Na dehydrocholate (relatively non-toxic) intravenously after the maximum spontaneous pressure has developed, a further increase of 10 to 30 mm. will be observed. The intravenous injection of 1 to 5 mgm. doses of secretin no. 372 caused the following effects: 1, it increased the rate of development of pressure. 2, if injected after the maximum spontaneous pressure has developed it either increased the pressure by 5–15 mm. of $CH\,Br_3$ (observed in 3 experiments), or retarded the process of resorption (observed in 2 experiments).

In connection with the foregoing experiments on the secretion of bile and the development of bile pressure we have used repeated doses of secretin no. 372. The intravenous injection of ten times as much Na cholate is without demonstrable effect. In our hands the greatest increase in the rate of bile flow from secretin stimulation has been from a basal rate of 0.128 cc. per minute to 0.278 cc. per minute, an increase of 117 per cent. This increased rate was maintained for over two hours by injecting secretin at 20 minute intervals. The injection of the same amounts of Na cholate did not alter the spontaneous rate of bile flow. However, the intravenous injection of 0.5 gram of bile acids increased the rate from 0.09 cc. per minute to 0.94 cc. per minute, an increase of over 1000 per cent, which effect lasted for over two hours.

In small doses secretin is a greater stimulant to the liver than are similar amounts of bile salts. However, it is impossible by increasing the dose of secretin to cause the marked outpouring of bile observed after large doses of bile salts.

Effect of secretin on the rate of bile flow in eviscerated animals. Mellanby (1928b) claims to have found that secretin no longer provokes a flow of bile after ligation of the pancreatic vein, and proposed the theory mentioned previously. We have removed the entire pancreas from dogs and have found that they respond quite normally to the intravenous injection of secretin. Mellanby has removed a portion of the intestine and found

it to have no marked effect upon the response to secretin. We wished to make sure that metabolites from none of the abdominal viscera were involved and prepared eviscerated animals for the study. The entire intestine, pancreas and spleen were removed. All of the blood vessels to the stomach were ligated. In one of our experiments there was no spontaneous flow of bile. The intravenous injection of 2 mgm. of secretin caused 11 drops of bile to flow and one-half hour later a similar injection caused 9 drops of bile to flow. One would not expect a normal reaction due to the trauma, shock and the loss of portal blood to the liver.

In two successful experiments we have re-established a blood flow through the portal system. The portal vein was anastomosed with the inferior vena cava by means of a Crile cannula. In one experiment the spontaneous flow of bile after the anastomosis was ½ drop per minute, then 4 mgm. secretin were injected; the flow increased to almost 2 drops per minute at the end of 10 minutes and then slowed down. In 35 minutes there were 30 drops of extra bile secreted due to the secretin. The other experiment was of the same general character and results.

These experiments, we believe, are to be considered proof that the cholagogue activity of secretin is not due to metabolites arising in the abdominal viscera, but rather to a direct effect upon the liver. The effect upon the liver may be due to a direct stimulation of the hepatic cells or to local circulatory changes. In view of the fact that vasodilatin free secretin has been shown to have no local vasomotor effects on the kidney and pancreas, it did not seem probable that the liver would be so affected.

The effect of secretin on the circulation of the liver. Dr. F. R. Griffith (1930) permitted us to use his liver plethysmographs for use with cats. We have used his technique in these experiments. The diaphragm of our tambour was made of condom rubber. Figures 1 and 2 show the tracings obtained from cats. We have injected peptone and adrenalin as well as secretin to show the effects upon the liver volume. In figure 2 the tambour was increased in sensitivity by extending the writing point connected through an extra lever, so that minor fluctuations in liver volume might be observed. The sample of secretin no. 413 is the best product we can make. It is vasodilatin free and 0.1 to 0.2 mgm. produces a copious secretion from the dog's pancreas. Our injection of 10 mgm. of that product in the cat represents more than 200 secretory doses. Should the cholagogue effect be obtained by virtue of circulatory changes in the liver, that dosage certainly should be sufficient to cause an observable effect on the liver volume. However, it will be noted (fig. 2) that no change in the liver volume was observed.

We have conducted some experiments on dogs having chronic biliary fistulae. The common bile duct was ligated and cut, a gold cannula was sewed into the gall bladder and that organ stitched to the abdomen. The

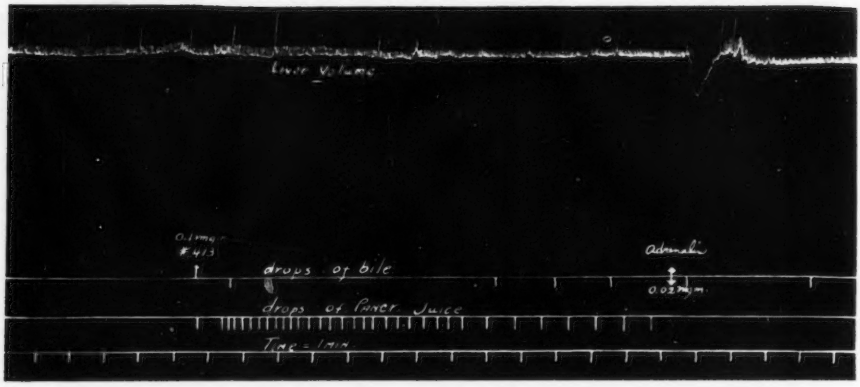

Fig. 1. The effect of an intravenous injection of purified secretin on the secretion of pancreatic juice, of bile and on the volume of the liver.

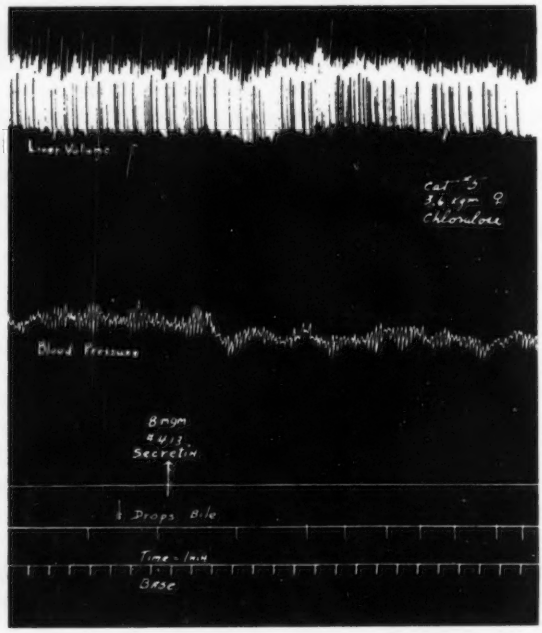

Fig. 2. The effect of approximately 200 secretory doses of purified secretin on the volume of the liver. An extra sensitive tambour was used in this experiment.

cannula was brought out through a small incision about two inches lateral to the main incision. Table 2 shows the results of the administration of 100 cc. of 0.35 per cent HCl by stomach tube and the intravenous injection of secretin in such an unanesthetized dog, 18 hours after its last meal.

It would seem from these observations that there is a compensatory diminution in the quantity of bile secreted after secretin stimulation. We have observed that the administration of secretin often does not augment the flow of bile in fistula dogs, during the first few hours (6–12) after a meal. No doubt this is because sufficient secretin is being absorbed to produce the maximal secretion from that mechanism. It is possible that Takacs used recently fed animals, and, therefore, did not observe the cholagogue effect of secretin. We have maintained an increased secretion

TABLE 2

The effect of secretin and HCl on the rate of bile flow from chronic fistula dogs

30 MINUTE PERIODS	EXPERIMENT I		EXPERIMENT II		EXPERIMENT III	
	Injections	Bile	Injections	Bile	Injections	Bile
		cc.		cc.		cc.
1	Control	1.8	Control	1.5	Control	1.9
2	Control	1.9	Control	2.1	Control	2.0
3	Control	2.6	Secretin 5 mgm.	2.6	Control	1.8
4	Control	2.1	Control	0.5	100 cc. of 0.35%	2.8
5	Control	1.8	Secretin 5 mgm.	3.0	HCl by stom-	2.9
6	Control	1.7	Control	0.7	ach tube	2.0
7	Secretin 5 mgm.	3.4	Control	1.6		1.5
8	Secretin 5 mgm.	4.3				1.5
9	Secretin 5 mgm.	4.7				
10	Secretin 5 mgm.	3.9				
11	Control	2.0				
12	Control	1.5				
13	Control	1.4				
14	Control	1.4				

of bile over periods as long as 6 hours. This indicates that the response of the liver to secretin does not become fatigued easily.

We have made many analyses of bile collected in these long experiments and have found that the "secretin bile" contains a lower concentration of bile acids than the spontaneously secreted bile. Our figures show that the total bile acids secreted per period are increased by secretin but not in proportion to the increase in volume. Therefore our data do not permit the statement of, but rather the suggestion, that secretin is a cholorrhetic.

In view of the fact that all measures which we have employed to inactivate the secretagogue activity of secretin also inactivates the cholagogue activity, it would seem very probable that the two activates are brought

about by the same agent. This view is supported by the observation that the two activities run parallel in all secretin preparations examined by us. Further support is to be found in the effect of the application of dilute hydrochloric acid to the duodenum as compared with the effect of intravenous injection of secretin, which comparison indicates that the acid provokes the flow of bile by increasing the absorption of secretin.

Although we are aware that certain hormones seem to bring about physiological activities in a given organ, indirectly by affecting some other more remote tissue, we believe our experiments express valid evidence that secretin per se is the agent stimulating the flow of bile. We believe the augmentation of the flow of bile is due to a direct stimulation of the hepatic cells and not to local or general circulatory changes. We have used a most sensitive method and certainly have been able to detect no important changes in liver volume even after administration of over 200 secretory doses of secretin.

CONCLUSIONS

1. The cholagogue action of secretin is not due to metabolites arising from the pancreas or other abdominal viscera.

2. Secretin, per se, stimulates the hepatic cells resulting in an augmentation in the flow of bile, which in acute and chronic experiments of 8 to 10 hours in length, contain lower concentrations of the bile salts.

3. Secretin does not cause circulatory changes in the liver as measured by the liver-volume method.

The authors wish to express their thanks to Dr. F. R. Griffith for the use of his liver plethysmograph, and to Messrs. Scott, Fradkin and Bolonik for valuable assistance given us.

BIBLIOGRAPHY

BAYLISS, W. M. AND E. H. STARLING. 1902. Journ. Physiol., xxviii, 325.
WHIPPLE, G. H. 1922. Physiol. Reviews, ii, 440.
MELLANBY, J. 1928a. Journ. Physiol., lxvi, i.
1928b. Journ. Physiol., lxiv, 331.
LUETH, H. C. AND G. KLOSTER. 1928. This Journal, lxxxv, 389.
STILL, E. U. 1930. This Journal, xci, 405.
TAKACS, L. T. 1928. Zeitschr. f. d. ges. Exp. Med., lxii, 114.
GRIFFITH F. R. AND F. E. EMERY. 1930. This Journal, xcv, 20.

THE EXCRETION OF URINE IN THE DOG

II. The Urea and Creatinine Clearance on Cracker Meal Diet

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This paper is an examination of urea and creatinine clearance in dogs maintained upon a cracker-meal and fat diet poor in protein, in comparison with our previously recorded observations on dogs maintained upon a mixed diet rich in protein (Jolliffe and Smith, 1931).

It is well known that the rate of excretion of all urinary constituents is affected by drugs, food, etc., when these are administered during or shortly before the period of observation. Because of this fact it is necessary to maintain certain standard conditions in experiments in which it is desired to compare renal function in various individuals or in the same individual at different times. Addis and Drury (1923) showed that mixed meals, milk, glutamic acid, etc., increased the rate of urea excretion in man in relation to the blood urea under conditions which were otherwise controlled and uniform. The increased renal activity was apparently attributable to protein, because glucose was without effect. Addis and Drury only reported experiments in which the meal or the administered substance was given during the period of observation; they did not look for more or less persistent effects of various maintenance diets. We had no reason to suspect and we were rather surprised to find, that dogs kept upon a protein-poor diet showed a marked decrease in renal activity in respect to the rate of excretion of both urea and creatinine, as compared with dogs kept upon mixed or protein-rich diets.

Methods. Except for the diet, the following experiments were performed in the same way as those previously reported by us. The cracker-meal diet consisted of 100 grams of cracker meal, 30 grams of lard and 30 grams of cane sugar moistened with warm water and well mixed. After the first few days the dogs consumed all their food; there was never any gastro-intestinal disturbance and they held their weight well for one to two months. It is estimated that the protein content of this diet was 9.6 grams per day. The mixed diet contained 55 to 110 grams, and the meat diet about 180 grams of protein per day.

All experiments were performed 16 to 18 hours after the last meal.

Urea and creatinine clearance on cracker-meal diet. Table 1 contains a series of observations on one dog (no. 18) showing the effect of diet upon the urea and creatinine clearance. The first group of observations (expt. 50) was made on the dog after it has been on a meat diet for two days. Urea and creatinine clearances were subsequently observed after five days of fasting (expt. 51); after 2 days on meat (expt. 52); after 6 days of fasting (expt. 53); after 9 days on cracker meal (expt. 54); after 2 days (additional) on cracker meal (expt. 55); after 3 days on meat (expt. 56); after 11 days additional on meat (expt. 57). The dog was then rested for eight weeks while feeding upon a mixed diet (expt. 70); expt. 76 was after 5 days on cracker meal and expt. 77 was after 2 days on meat.

The urea clearance (considering only those values where the rate of urine flow was above 0.4 cc. per minute) began at an average of 61.7 on the meat diet and dropped to 28.9 during fasting; it rose to 60.9 again on meat and fell to 32.8 during the second fasting period; it remained practically constant at 33 during the time that the dog was fed cracker meal but rose again during the first 3 days on meat, and it was still higher (70.8) after an additional 11 days on meat; it fell to an intermediate level during the feeding of a mixed diet (45), to a low level (35.2) on cracker meal and finally rose again (54.9) after 2 days on meat.

It will be seen that the urea clearance may be increased more than 100 per cent by changing from fasting to a pure meat diet, and that the feeding of maintenance quantities of cracker meal, fat and sugar do not increase the clearance significantly above the fasting level. These results are consonant with our observations on two other dogs, only one of which will be reported here (no. 20; see below under *augmentation limit*). It is obvious that if one wishes to compare the renal activity of various dogs, or of the same dog at various times, the diet must be carefully controlled.

In one case urea was administered parenterally in order to raise the blood urea (expt. 55). The urea clearance was not changed (31.8) as compared with observations (expt. 54) made 2 days previously (32.5). This experiment is valuable in showing that the low clearance observed on the cracker-meal diet is not due to error in the analysis of the low blood ureas.

It will be noted that the urea and creatinine clearances are affected in a parallel manner by diet. The urea : creatinine ratio in dogs maintained on a mixed diet (Jolliffe and Smith, 1931) averaged 0.533 $\pm$0.073, while this ratio when maintained on a cracker-meal diet averaged 0.515 $\pm$0.037, a lowering of the ratio of 0.018 $\pm$0.015 which is statistically not significant. This fact may indicate that the changes in renal activity which are induced by diet are primarily referable to changes in the quantity of glomerular filtrate per unit time rather than to changes in the quantity of these substances secreted. If this interpretation is accepted, it would appear that nearly half of the filtered urea is normally reabsorbed. But we would

TABLE 1

Effect of diet upon urea and creatinine clearance

Dog 18, surface area = 0.77 square meter.

EXPERIMENT NUMBER	V URINE VOLUME	UREA		UREA CLEARANCE	CREATININE		CREATININE	UREA: CREATININE RATIO	DATE AND REMARKS
		Serum	Urine	$\frac{UV}{S}$/S.A. Maximum	Serum	Urine	$\frac{UV}{S}$/S.A.		
	cc. per minute	*mgm. per 100 cc.*	*mgm. per 100 cc.*		*mgm. per 100 cc.*	*mgm. per 100 cc.*			
50	8.030	34.8	240	71.9					3-5-31
	5.480	34.8	317	63.6	43.0	556	91.9	0.692	Meat diet since
	2.930	32.3	491	58.0	41.4	977	89.5	0.648	3-2-31
	0.740	28.4	1,610	53.2	32.9	2,982	87.2	0.610	
51	0.419	27.2	1,565	31.8					3-9-31
	0.506	27.2	1,092	26.0	61.4	5,597	64.3	0.404	Fasting since 3-5-31
	0.349	26.8	1,465	24.8	55.1	6,672	55.2	0.449	
	0.249	27.2	2,150	25.3	42.2	7,670	58.7	0.432	
52	1.042	56.5	2,931	70.0	33.6	2,641	106.5	0.657	3-11-31
	0.703	55.5	3,355	55.2	33.6	3,561	96.7	0.581	Meat diet since
	0.543	51.9	4,212	57.4	34.4	4,065	83.3	0.688	3-9-31
53	0.181	23.5	2,967	(29.5)*					3-16-31
	0.333	23.5	1,511	27.7	44.2	5,362	52.6	0.527	Fasting since
	0.567	24.3	1,170	35.4	53.7	5,020	68.9	0.514	3-11-31
	2.333	25.0	250	30.2	59.6	1,239	63.0	0.476	
54	0.629	11.6	367	25.9	73.9	5,565	60.3	0.430	3-25-31
	0.663	11.3	386	29.5	65.6	4,610	60.3	0.489	Cracker-meal diet
	2.270	10.4	118	33.4	61.5	1,194	58.3	0.573	since 3-16-31
	3.600	10.9	83	35.7	53.7	717	62.5	0.571	
	3.262	10.8	95	37.2	47.6	946	77.9	0.477	
55	0.828	71.0	2,002	30.3	45.7	2,601	59.0	0.514	3-27-31
	0.796	81.8	2,292	29.0	46.9	2,822	62.4	0.465	Cracker meal diet
	1.162	89.6	1,895	31.9	52.4	2,122	61.1	0.522	since 3-16-31
	3.10	87.2	750	34.5	52.4	750	57.6	0.599	Urea admin.
	3.50	86.8	637	33.4	52.4	686	59.6	0.561	
56	0.417	32.1	2,175	37.2	48.9	5,760	64.0	0.581	3-30-31
	0.391	30.3	2,258	37.8	46.8	6,420	67.2	0.562	Meat diet since
	0.434	29.9	2,420	45.7	42.2	5,369	71.6	0.638	3-27-31
	0.400	29.3	2,220	39.5	38.3	4,740	63.9	0.615	
	0.375	28.8	3,031	51.1	32.6	5,561	83.0	0.616	
	0.317	25.3	3,210	52.2	20.3	4,740	95.9	0.544	

* Standard clearance = 69.6

TABLE 1—*Concluded*

EXPERIMENT NUMBER	V URINE VOLUME	UREA		UREA CLEARANCE	CREATININE		CREATININE	UREA: CREATININE RATIO	DATE AND REMARKS
		Serum	Urine	$\frac{UV}{S}$/S.A. Maximum	Serum	Urine	$\frac{UV}{S}$/S.A.		
	cc. per minute	*mgm. per 100 cc.*	*mgm. per 100 cc.*		*mgm. per 100 cc.*	*mgm. per 100 cc.*			
57	0.642	43.0	3,435	66.5	39.9	5,215	109.0	0.610	4–7–31
	0.719	41.7	3,390	75.8	32.3	4,480	129.3	0.587	Meat diet since
	2.330	38.8	955	74.5	29.9	1,247	126.2	0.589	3–27–31
	7.660	36.8	263	71.1	29.9	352	116.9	0.608	
	10.450	38.6	193	68.0	26.1	224	120.8	0.563	
70	2.95	37.2	472	48.5					5–29–31
	5.08	35.1	261	49.9					Mixed diet since
	4.82	33.1	257	48.3					4–7–31
	2.13	32.1	491	42.3					
	0.9	32.1	988	36.0					
	0.238	31.5	2,504	25.0					
76	1.345	20.0	358	31.3	24.5	943	67.2	0.466	6–8–31
	0.404	20.8	1,465	36.9	25.2	3,430	71.4	0.517	Cracker diet since
	0.310	21.7	1,924	34.7	25.6	4,242	67.8	0.497	6–2–31
	0.409	23.6	1,665	37.4	25.6	3,228	66.9	0.559	
77	1.656	35.6	826	50.0	44.5	1,870	90.2		6–10–31
	0.684	36.8	2,430	58.7	40.7	4,200	91.8		Meat diet since
	3.755	35.6	386	52.8	36.6	672	89.6		6–8–31
	2.725	32.9	496	53.3	32.6	838	90.9		
78	3.620	41.2	448	51.3	37.1	677	85.7	0.599	6–15–31
	5.260	41.1	316	52.6	36.5	547	102.5	0.513	Meat diet since
	4.760	41.5	348	51.3	33.6	564	103.8	0.494	6–8–31
	1.000	39.9	1,250	54.3	33.1	2,062	81.0	0.672	

call attention again to the fact that this apparent reabsorption of urea shows no correlation with the urea concentration of the urine, as one would expect if the reabsorption were due to passive diffusion from the highly concentrated tubular urine or from the bladder. In fact, the fraction of apparent reabsorption may be the same whether the urine urea is 200 or 3000 mgm. per cent (expt. 57).

We recognize that the administration of creatinine may influence the excretion of urea, but do not believe that such is the case. No difference was observed in the urea clearance with and without creatinine in one dog previously reported (Jolliffe and Smith, 1931), and we have further observations on this point which will be described at another time.

TABLE 2

Effect of diet upon the urea clearance at low rates of urine flow

Dog 22, surface area = 0.73 square meter.

EXPERIMENT NUMBER	V URINE VOLUME	UREA		UREA CLEARANCE		DATE AND REMARKS
		Serum	Urine	$\frac{UV}{S}$/S.A. Maximum	$\frac{u\sqrt{V}}{S}$/S.A. Standard	
	cc. per minute	*mgm. per cent*	*mgm. per cent*			
61	0.656	16.8	1,184	63.7		4-28-31
	3.75	16.7	211	65.4		Mixed diet
	5.00	16.4	158	66.1		
	5.80	17.1	141	65.5		
64	0.066	7.24	2,430	29.7	118.0	5-2-31
	0.082	7.20	1,880	29.5	102.6	Cracker meal diet
	1.93	6.42	76.4	31.1		since 4-28
65	0.069	11.2	2,056	17.1	66.1	5-9-31
	0.054	11.2	2,910	19.0	111.0	Cracker meal diet
	0.573	10.8	517	37.1		since 4-28-31
67	0.252	9.9	860	29.7		5-16-31
	2.3	9.8	85	29.0		Cracker meal diet
	0.591	9.8	371	30.3		since 4-28-31
72	0.069	9.7	1,980	19.4	73.7	6-1-31
	0.064	9.7	1,935	17.6	67.7	Cracker meal diet
	0.600	10.8	480	36.4		since 4-28-31
	2.90	10.8	93	34.1		
73	0.042	19.5	4,285	12.7	59.7	6-3-31
	0.038	19.3	4,228	11.3	56.6	Cracker meal diet
	0.041	19.0	3,875	11.3	55.1	since 4-28-31
	0.043	18.8	3,941	12.2	58.0	
75	0.073	13.2	2,684	20.3	75.1	6-5-31
	0.049	13.1	2,968	15.2	69.0	Cracker meal diet
	0.083	12.9	2,330	20.5	95.9	
	0.137	12.5	1,745	26.2	70.5	
	0.677	12.5	428	31.8		
80	0.142	39.2	7,060	35.0	92.9	6-18-31
	0.542	37.0	2,665	53.8		Meat diet since
	4.150	33.1	342	58.5		6-10-31
	5.470	31.6	222	52.7		
	4.250	31.0	278	52.2		

Augmentation limit on cracker-meal diet. Austin, Stillman and Van Slyke (1922) have shown in man that when the urine volume falls below a certain value (augmentation limit) the rate of excretion of urea is proportional to the square root of the volume of urine. This relationship appears to hold true in the dog when maintained upon a mixed diet (Jolliffe and Smith, 1931). It is therefore of both practical and theoretical importance to determine if an augmentation limit exists in dogs fed upon cracker-meal.

In nearly all the observations on dog 18 (table 1) the urine volume exceeded 0.4 cc. per minute, which was the augmentation limit noted by us on dogs fed on a mixed diet. We have therefore included in table 2

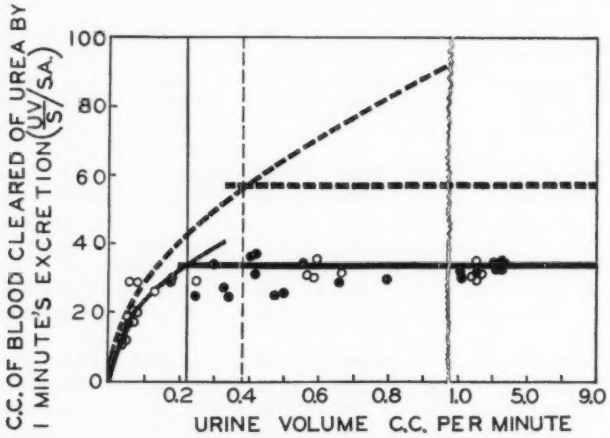

Fig. 1. The urea clearance in relation to urine volume in dogs fed a cracker meal diet (solid lines) in comparison with dogs fed a mixed diet (broken lines) previously reported (Jolliffe and Smith, 1931). The solid curved line is calculated according to $\frac{U\sqrt{V}}{S}/S.A. = 71.2$ and intersects the solid horizontal line $\left(\frac{UV}{S}/S.A. = 33.6\right)$ at the augmentation limit, through which the solid perpendicular line is drawn. The circles are observations on dog 22; the dots are observations on dog 18.

a series of observations on another dog (no. 22) in which the urine was both below and above the augmentation limit. The urea clearances from dog 22, and also from dog 18, are given graphically in figure 1. It will be seen that when the urine volume falls below 0.2 cc. per minute (as shown by our subsequent calculations) the excretion of urea falls rapidly with decreasing urine volume. Our data are insufficient to establish whether or not $U\sqrt{V}/S$ exactly defines the urea excretion below this augmentation limit, but they leave no doubt that an augmentation limit exists on a cracker-meal diet as well as on a mixed diet. The curves in figure 1 are calculated as follows: the maximal clearance is taken as the average of all observations above 0.4

cc. per minute urine volume; the standard clearance is taken as the average of all observations below 0.1 cc. per minute urine volume (omitting the first two observations in expt. 64).

From these data, we obtain from a cracker meal diet,

$$\text{Standard clearance, } \frac{U}{S}\sqrt{V}/S.A. = 71.2$$

$$\text{Maximal clearance, } \frac{UV}{S}/S.A. = 33.6 \pm 3.1$$

$$\text{Augmentation limit} = 0.22 \text{ cc.}$$

It appears that the augmentation limit decreases in proportion to the maximal clearance.

The number of observations reported here are less than were reported for a mixed diet; nevertheless it appears that the standard deviation in the maximal clearance is significantly less on the cracker-meal diet (± 3.1 in 23 observations) than on the mixed diet (± 11.9 in 66 observations). Both groups fulfill statistical requirements in that about two-thirds of all observations fall within the standard deviation. So it would appear that not only a lower clearance, but a more uniform clearance is obtained on a cracker-meal diet than on a mixed diet. This is further illustrated by the fact that dog 22 shows a maximum clearance about 66 and dog 18 about 45 on a mixed diet, while they both drop to an average of 33.6 ± 3.1 on cracker-meal.

SUMMARY

The urea and creatinine clearances, as determined 16 to 18 hours after the last meal, are reduced to low levels in dogs which are either fasted or fed upon a protein-poor diet composed of cracker meal, lard and sugar. The substitution of a mixed diet produces a marked elevation of the urea and creatinine clearances, and the substitution of an exclusive meat diet increases both clearances more than 100 per cent. This effect is not related to the absolute amount of urea excreted. The urea clearance shows more uniformity on a low protein cracker-meal diet than on a mixed diet.

The augmentation limit appears to be lower on a cracker-meal than on a mixed diet in proportion to the lowering of the maximal clearance.

BIBLIOGRAPHY

Jolliffe, N. and H. W. Smith. 1931. This Journal, xcviii, 572.

Addis T. and D. R. Drury. 1923. Journ. Biol. Chem., lv, 629.

Austin, J. H., E. Stillman and D. D. Van Slyke. 1921. Journ. Biol. Chem., xlvi, 91.

THE IRRITABILITY CHANGES IN NERVE IN RESPONSE TO SUBTHRESHOLD INDUCTION SHOCKS, AND RELATED PHENOMENA INCLUDING THE RELATIVELY REFRACTORY PHASE[1]

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In 1883 Wedenski showed that stimuli sufficing to produce contraction of a muscle, indirectly stimulated through its nerve, may fail when they are repeated at certain relatively rapid rates. Proposed explanations of this phenomenon have failed to carry conviction until Kato and collaborators in 1929 ascribed it to an "after-effect", a brief period of lowered irritability, left by an ineffective nerve impulse or by an artificial stimulus of less than threshold value. In confirmation of Gildemeister (1908), the Japanese investigators found that, following a subthreshold induction shock, the local irritability of nerve at first is raised momentarily (they were dealing here with the "summation interval" of previous investigators) and then lowered. Though the values they give, namely, 1 to 2 σ and 10σ, for the periods of enhanced and depressed irritability, respectively, are somewhat larger than those previously found by Gildemeister, 0.4 and 3σ, there is every reason for believing that both investigations deal with the same phenomenon. Gildemeister ascribed the irritability changes to an "inertia effect" initiated by but outlasting the shock. Levinsohn (1910) observed the same reduction in irritability in muscle directly stimulated and, without specifying any reason for so doing, refers to it as the relative refractory phase.

The present investigation, in its inception a study of the Gildemeister phenomenon, was undertaken in the hope of obtaining information relative to the nature of the mechanism underlying it. It proved a simple matter to verify Gildemeister's observations. Then evidence was obtained indicating that the phase of depressed irritability is a state of postcathodal depression left by the subthreshold induction shock, and the investigation, therefore, was extended to include, in addition to effects produced by subthreshold induction shocks, a consideration of certain comparable effects

[1] The results on which this paper is based were presented at the meeting of the American Physiological Society, April 10, 1931 (This Journal, 1931, xcvii, 519).

incidental to the action of subthreshold constant and other currents. Evidence was thus obtained that was confirmatory of the conclusions derived through observations on induction shock activation, and along with this, considerable information that is not directly related to the original problem. Therefore, most of the results derived through the use of the constant current for activation can best be considered separately and will be included in another paper.

METHODS IN GENERAL. The index to the response of nerve in studies of this character almost invariably has been the height of contraction of muscle indirectly stimulated. The measure used in the present investigation has been the electrical response of the nerve itself, visualized, after amplification, by the cathode ray oscillograph. Among the advantages possessed by this method may be mentioned 1, the immediate presentation of deflections to the eye; 2, its high precision, and 3, the unfatiguability of the observed response at the rates of stimulation employed. 1. During all of the manipulations, preliminary and final, one sees on the screen of the oscillograph not only the action potential itself, but also, qualitatively, the time relations it bears to the applied electrical stimuli and polarizing currents, recognizable by their electrical artifacts. It thus becomes a simple matter to regulate stimulation strength and adjust time intervals. When the desired adjustments, thus controlled, have been made dots may be placed on the screen to mark, for example, the beginning and end of a constant current, of a testing induction shock, the front, crest and end of an action potential, and what not. Required measurements can then be made at once on the tube, or the points may be transferred to cellophane for preservation and subsequent measurement, or a contact print can be made on film of the picture presented by the screen of the tube. 2. When the conditions are favorable, measurements can be made with a fair degree of accuracy of time intervals as short as 0.01σ. Furthermore, since maximum action potentials can, if necessary, be given an effective amplitude of over two meters, threshold values can be determined with the greatest of precision; and the height produced by experimental variation in the number of contributing axon potentials or by other causes, usually can be given a range that reduces to a very low value the experimental error of measurement. 3. In most of the experiments the nerve was stimulated practically continuously, sometimes for hours, at the rate of about once a second. It was thus maintained in a steady state of activity for the quantitative observations that could be made only occasionally after the desired conditions had been supplied.

The nerve studied has been the sciatic of *Rana pipiens*. The stimulating electrodes always were nonpolarizable and were either of the Lapicque or calomel (Erlanger, Bishop and Gasser, 1926) types. The lead electrodes were either large silver wires or calomel half cells. The nerve was mounted

in a moist chamber that could be kept at a constant temperature; except where otherwise stated it was at the temperature of the room which ranged in one series of experiments (summer) between 26 and 28°C., in another (winter) between 18 and 24°C. During the latter period the chamber in many of the experiments was held at about the temperature of the water of the aquarium in which the frogs lived, namely, 10 to 14°C.

Except where otherwise stated the first or *sensitizing* shock, condenser discharge, or constant current was below the threshold, usually by a known percentage. The irritability of the nerve was always determined by the *test* shock, a break induction shock with cathode proximal with respect to the lead. For the sake of brevity such a shock will be called a cathodal shock. To test irritability one of several methods was used. Quantitative data may be obtained by varying a known primary current so as to keep the action potential at a chosen constant height. Heretofore the height usually chosen in such experiments has been threshold, and we have made some determinations by this threshold method. It has been found, however, that the method can be made much more precise by using a stimulus that lies in the range where variations in strength effect the widest changes in action potential amplitude, namely, in the low submaximal region. This we shall designate the constant height method. In practise a line parallel to the base line is drawn on the face of the tube at the selected height and the primary current needed to put the crest of the action potential in that line is determined. With changing shock spacings the action potential moves, of course, into different positions along this line. Quantitative data expressing the relation of stimulus strength to magnitude of response, threshold or constant height, also can be obtained simply by referring readings of coil separation to a chart we have made, in effect, by plotting coil separation of the testing inductorium against height of the induction shocks directly recorded with the cathode ray oscillograph.

When the irritability range in an experiment does not exceed the limits subtended by threshold and maximum the amplitude of the action potential produced by a shock of constant strength varies, though not exactly proportionally, with the irritability of the nerve. Though curves based on data thus obtained are not superimposable on those made by the preceding methods all, nevertheless, have the same general form and agree exactly as regards temporal positions of phase changes. This method of gauging irritability by amplitude of response has certain very decided advantages, of which may be mentioned speed and ready verifiability. The amplitude-time curves can be traced on the face of the tube by placing a dot on the crest of the action potential in as many positions as may be necessary. The curve thus outlined can be verified over and over again by carrying the action potential backwards and forwards through the time range as often as may be necessary to attain the desired accuracy.

When dealing with alterations in irritability of small magnitude, such as obtain during the negative phase of the Gildemeister phenomenon, the nerve currents usually were amplified about 100,000 times (four-panel amplification). At this amplification a maximum action potential would have a height of more than two meters were it possible to record it; but for these purposes the test shock was given a strength that would produce a "normal" action potential about 2 to 3 cm. high. With three-panel amplification, giving maximum action potentials 8 to 10 cm. high, these threshold action potentials produce imperceptible deflections. Though the smaller irritability changes are more readily measured by the amplification given by four panels, they, nevertheless, are demonstrable also when amplified by three panels (about 8000 times).

Since even slight variations in shock strength and form affect very materially the height of a threshold action potential that is amplified 100,000 times, it was necessary to have for these experiments uniform induction shocks. To attain them, we have, besides resorting to devices that minimize sparking, such as the use of relatively high primary resistances and, when necessary, a shunt around the primary key, tried quite a variety of switches. Our best switch consists of a straight, light, but stiff, ivory spring tipped with very light steel to prevent frictional wear. A platinum point on the ivory spring is pressed by the cam of our rotating interrupter against another point firmly held above it in a screw, by means of which the contact pressure is adjustable. For each special purpose it was necessary to modify the circuit used; those that require description will be considered each in its proper place.

IRRITABILITY CHANGES INITIATED BY A SUBTHRESHOLD INDUCTION SHOCK. The investigation was begun by repeating the Gildemeister experiment of testing with a submaximal shock of constant strength the changes in irritability incident to a subthreshold shock. The type of circuit used for the purpose is shown in figure 1. The resulting irritability changes (see figs. 2, 3, 4, and 6) may for convenience of treatment be divided into the positive phase, or summation interval, and the negative phase, or Gildemeister phenomenon proper.

The summation interval: The maximum response to the testing shock is coincident with the beginning of the sensitizing shock. From this peak the curve falls off rapidly but with a slightly retarding rate as the testing shock is moved later, the summation interval ending under normal circumstances without any obvious discontinuity at the point, by definition, where the negative phase begins. The duration of the summation interval has ranged between about 0.2 and 1.7σ, the variations usually being referable to specifically imposed conditions, such, for example, as shock duration and strength, temperature and polarization. It is necessary to say, however, that occasionally verifiable durations have been found that have been out of accord with the rule; we are unable to account for these exceptions.

Does shock duration determine the summation interval? A factor that might be expected to exert an influence on the duration of the summation interval is the *duration of the sensitizing shock*. Indeed, it was very necessary to ascertain at the outset whether shock duration does not actually account entirely for the summation interval. Previous investigators have discarded this possibility on the basis either of the assumed brevity of the shocks they used (e.g., Gildemeister, 1908) or (and) of the differences in the summation intervals of different tissues activated by what they regarded as the same shock (e.g., Lucas, 1910). But coils, such as they used, so built as to yield brief shocks, can under circumstances deliver shocks long enough quite to extend through the summation interval of nerve, certainly, and possibly even of voluntary and heart muscle. Second-

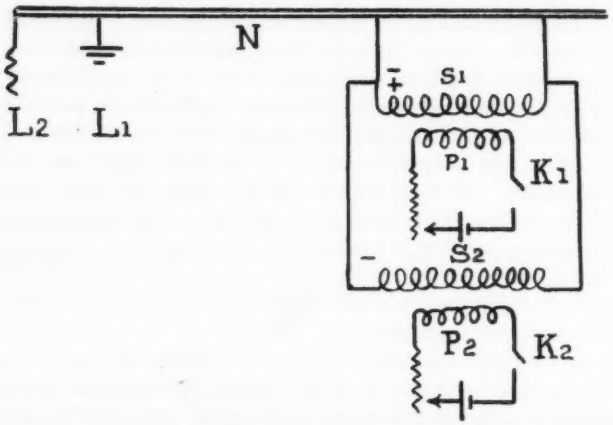

Fig. 1. Arrangements for recording the conducted action potential started by a cathodal testing shock (from S_2) after sensitization by either a cathodal or an anodal shock from S_1. N, nerve; L_1, ground lead; L_2, grid lead into amplifier from killed end.

ary resistance, for example, affects shock duration very decidedly and the lower interelectrode resistances that must have obtained where muscle took the place of nerve undoubtedly must have prolonged very materially the shocks which otherwise would have been alike. It will be shown that these differences can be sufficient to have produced the summation intervals of nerve and muscle that have been recorded by previous workers. Gildemeister believes that the possibility of physical superposition of currents, sensitizing and testing, was controlled in his experiments by the use as sensitizing current of a condenser discharge of such a duration that the deflections of a ballistic galvanometer in the place of the nerve *did not change in amplitude with the alteration in the interval between shocks.* But condenser discharges, like some induction shocks, end asymptotically,

that is to say, at an indeterminate time; and a very small terminal fraction of the total energy of the discharge, one so small as to be without appreciable effect on the throw of a galvanometer, could nevertheless very materially affect the irritability of nerve. Therefore, it still remains to be shown that summation interval and shock duration are not coterminal.

Summation of shocks from inductoria may be obtained by joining the secondary coils either in series, or as has generally been done, and as we have done, in parallel. In the case of the series arrangement, as used by Kato and others, one would expect to obtain summation of potentials as long as the shocks overlap, though we have not ascertained what actually happens under these circumstances. But coils in parallel might be expected to behave as do cells in parallel, and not sum their potentials. As a matter of fact they do. Direct Braun tube records of induction shocks from the coils arranged as we have used them (see fig. 1) show that the shocks sum practically perfectly and without other alteration in form. Furthermore, depending primarily upon the secondary resistance, the presence or absence of an iron core, or of a shunt in the primary circuit, the shock may have a variety of configurations and durations. Pending separate publication of observations on coil behavior, it must suffice here to call attention to certain type variations. The natural oscillation period of our secondary circuit is such that a half cycle lasts about 0.03 to 0.04σ. The air-cored coil with the usual secondary resistance of 10,000 to 30,000ω delivers but a single phase of an oscillation. Higher secondary resistances increase the number of oscillations and their symmetry around zero, complete damping requiring about 0.4σ when the secondary resistance is infinite. Insertion of the iron core and of a shunt around the primary terminals both prolong the secondary process and damp the oscillations which now distribute themselves along the decrementing secondary potential. With the secondary resistances usually obtaining in our experiments the iron-cored coil with a 40ω shunt around the primary terminals, delivered a shock lasting 0.2 to 0.6σ; and with the lowest secondary resistance tried (230ω) the shock lasted 8σ. In the experiments of this section one of the secondary coils is shunted by the other and, since the resistance of the secondary coil is 230ω, one might expect the shocks to be relatively long in duration when one only of the pair of coils is activated. As a matter of fact the parallel coil acts, presumably through its impedance, practically as an infinite resistance with respect to the coil it parallels and does not prolong shocks from the latter.

Under the circumstances, it obviously still remains to be ascertained experimentally whether the increase in irritability produced by a subthreshold shock from a "quick" coil outlasts the applied potential. The evidence that we have accumulated all indicates that it can. The shortest shocks we use last, say, 0.04σ, and these may give rise to summation inter-

vals lasting almost 2σ. It is scarcely conceivable that these shocks are prolonged beyond their measurable span by an undetectable asymptotic termination lasting through such a summation interval.

All ambiguity with respect to this question has been removed by an experiment in which the sensitizing coil was short-circuited in the interval between the termination of its shock and the end of the summation interval. For this experiment the usual circuit was changed by inserting a short-circuiting switch across the terminals of the sensitizing coil and a resistance of 100,000ω between this and the testing coil in order to reduce to an undetectable value the effect of the short circuit on the testing shock. The short circuit was closed 0.20σ after the break of the sensitizing coil. The summation interval of 0.67σ (18°C.) was unaffected by the procedure.

There are a number of other observations that are inconsistent with the view that the duration of the summation interval is determined primarily by the summation of shock potentials. For example, one cannot, on such a basis, account for the variations in the summation interval that accompany variation, 1, in duration, 2, in strength of sensitizing shock, or 3, in temperature of the preparation, or 4, polarization.

Does shock duration affect the summation interval? Shocks adequately brief and different in duration to supply the information needed to answer this question were obtained in one set of experiments merely by using at one time the air-cored, at another, the iron-cored coil. They were given the same physiological value as stimuli by reducing the primary current a given per cent below its threshold strength in each case, on the basis of the principle that the physiological action of shocks is proportional to their energy content. This, however, cannot be quite true when the shocks, as is the case here, differ in configuration. It is realized, furthermore, that the proportionality of energy content of shock to the primary current holds perfectly in the case of the air-cored coil only. The data from two of the experiments, which can be regarded as typical, are as follows: duration of summation interval, *a*, core in, 0.46σ, core out, 0.62σ; *b*, core in and out 0.78σ. In *b* the shock delivered by the iron-cored coil under the conditions of the experiment lasted 0.56σ; the shock from the air-cored coil under the same circumstances never has lasted over 0.1σ. In another experiment determinations were made of the irritability changes resulting from sensitization by an induction shock from an air-cored coil lasting 0.04σ and by a condenser discharge (others to be considered later) lasting 0.33 to 0.50σ (Braun tube measurements), both 80 per cent of threshold (12.8°C.). As may be seen in figure 2 the two irritability curves derived from the data of the experiment are identical.

The results obtained with these currents, which were brief relative to the summation interval, therefore, definitely indicate that varying the duration of relatively short sensitizing shocks of the same strength physio-

logically, is without effect on the duration of the summation interval. However, the agreement between experiments performed under apparently comparable conditions is by no means perfect. Possibly this variability, in part at least, is an expression of the uncertainties inherent in the principle employed in maintaining shock strength at comparable levels.

It is to be expected when sensitizing currents of comparable strengths are made long relative to the summation interval, that summation of potentials itself will prolong the period of increased irritability. The configuration of the irritability curves under these circumstances has been worked out in one experiment (fig. 2). The sensitizing currents (always

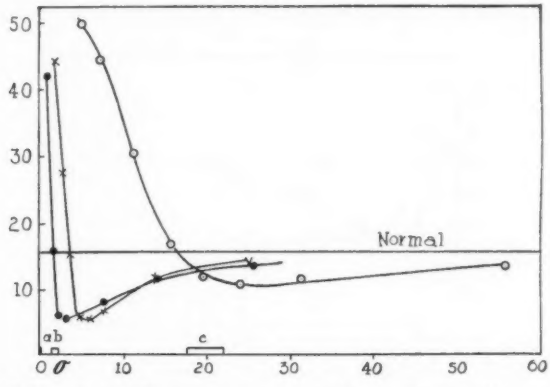

Fig. 2. (3/5/31) Irritability response initiated by a shock and by condenser discharges of different durations. 12.8°C.

Dots = Curve from shock lasting 0.04σ and from condenser discharge lasting 0.33 to 0.50σ (*a* on base line).

Crosses = Curve from condenser discharge lasting 1.40 to 2.31σ (*b*).

Circles = Curve from condenser discharge lasting 17.54 to 21.94σ (*c*).

Ordinates, amplitude of action potential; abscissae, time in sigmas.

80 per cent of threshold) were, in addition to the 0.04σ induction shock and the condenser discharge lasting 0.33 to 0.50σ, condenser discharges lasting, one, 1.40 to 2.31, and another 17.54 to 21.94σ, the figures given being the range of the limit of accuracy of measurement of the pictures of the discharges on the Braun tube. The period of increased irritability produced by the 1.40 to 2.31σ discharge outlasts the latter between 1.39 and 2.30σ, as nearly as can be determined; the discharge proper, therefore, is followed by a true summation interval of about the same duration as that produced by the briefer currents. The curve of irritability change produced by the slowest condenser discharge falls below the normal level apparently before the condenser potential reaches zero; seemingly in this case there is no

summation interval properly so-called. It is difficult, however, to exactly locate the end point of this slow discharge and we do not care to stress this relation, resting, as it does, on but a single reading and one that it is difficult to make with precision.

2. *Effect of shock strength.* The usual effect of increasing the strength of an induction shock is to increase the duration of the summation interval; this is seen in figure 3, where the summation interval is increased from about 0.25σ, when the strength of the sensitizing shock is 53 per cent of threshold, to 0.35σ at 76 per cent of threshold and to 0.53σ at 97 per cent of threshold (26°C.). Apparently the summation interval increases in duration more rapidly than the strength of the activating shock, but to ascertain the exact relationship between shock strength and summation

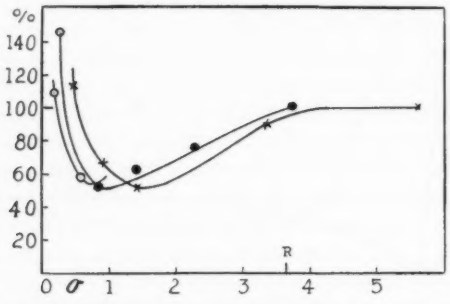

Fig. 3. (2/24/30) Irritability responses to cathodal sensitizing shocks; testing shock slightly superthreshold. 26.0°C.

Circles = Sensitizing shock 53 per cent of threshold.

Dots = Sensitizing shock 79 per cent of threshold.

Crosses = Sensitizing shock 97 per cent of the threshold.

Ordinates, per cent of normal amplitude. *R* indicates end of relatively refactory phase.

interval would require observations on cold nerve so as to provide a wider time range than obtained in the instance cited, and this has not yet been done. Since increasing the strength of the shock from an air-cored coil does not increase shock duration, this relation of shock strength to the summation interval supports the conclusion that the duration of the summation interval is independent of the duration of shocks when the latter are relatively brief. It also indicates that the initial local state produced by these subthreshold shocks does not obey the all-or-none principle.

3. *Effect of temperature.* The behavior of the summation interval in response to alterations in the temperature of the nerve also proves that the duration of the summation interval is determined by some process

initiated in the nerve by the shock; for temperature alters very materially duration of summation interval but only slightly duration of shock. It changes shock duration through the change in secondary resistance resulting from the alteration in the electrical conductivity of nerve by temperature. A 10° fall in temperature, for example, increases the nerve's resist-

TABLE 1

Some of the observations on the effect of temperature on the duration of responses

DATE	ORDER	TEMPERATURE	SENSITIZING SHOCK	SUMMATION INTERVAL		DEPRESSION PHASE		REFRACTORY PHASE
						Low	End	
			per cent	σ	Q_{10}	σ	σ	σ
6/23/30	1	27.0		0.4	2.05	1.0	4.27	3.94
	2	17.0	?	0.82	3.61	2.23	7.95	6.55
	3	10.5		1.87			16.02	±100.0
6/24/30	1	27.0		0.54	1.81			
	2	17.0		0.98	1.64(10°)			
	3	10.0–75	?	1.24	1.06		17.09	47.74
	4	25.0		1.13			3.26	2.63
6/25/30	1	27.7		0.89	1.39	1.25	5.89	5.09
	2	17.5		1.24		2.14	9.47	5.83(?)
	3	17.0	80	1.62	neg.	2.50	9.10	5.96(?)
	4	10.0		1.48		2.40	25.00	24.85
	5	10.5		1.75	1.7	3.71	16.78	33.49
	6	26.8		0.75		1.35	4.75	2.75
6/27/30	1	27.7	75	0.91	1.61			
	2	17.5		1.46				
6/30/30	1	28.0		0.95	1.48	1.31(?)	2.55	2.15
	2	17.7		1.41	1.00	2.91(?)	6.12	5.15
	3	11.0	75	1.40	1.44	2.69(?)	16.48	26.67(?)
	4	28.0		0.6				
7/ 2/30	1	25.7		0.78	1.55	1.06	3.80	3.80
	2	16.6		1.16		3.01(?)	8.72	9.47
	3	17.4	?	0.89	1.02	1.62	7.48	7.74
	4	9–10		1.02	1.35	3.01	21.11	40.75
	5	26.0		0.62		0.95	4.17	4.78

ance about 20 per cent. If the nerve's resistance at the higher temperature were 10,000ω and all other secondary resistance 20,000ω, the total secondary resistance would be increased by the temperature change from 30,000ω to 32,000ω, a change that would alter the duration of the shock inappreciably.

Some of our data on the effect of temperature on the changes in irritability of nerve responding to a subthreshold shock are collected in table 1.

Practically all of the experiments were started at room temperature and the temperature, therefore, first was lowered. Had the observations been limited to this first temperature span the interpretation of the data would have been simple; it would have been concluded that Q_{10} ranges between about 2 and 1.4, the higher figures being relatively infrequent. Other determinations, however, range from 1 or less to 3.6, a variation that is far beyond the range of error. These variations are not the result of failure to allow sufficient time for adaptation of the nerve to the new temperature, a half hour, at least, having been allowed to elapse between temperature changes. It is possible, on the other hand (Broemser, 1921), that these irregularities bear some relation to time of exposure of the

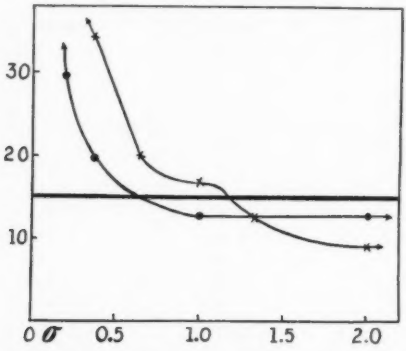

Fig. 4. (2/25/31) Irritability changes initiated by the same shocks from an air-cored coil, 89 per cent of threshold in both cases. 13.5°C.

Dots = In normal nerve.

Crosses = At the cathode of a nerve polarized with a constant current, 90 per cent of rheobase.

Ordinates, amplitude of action potential in millimeters. The heavy line marks normal height.

nerve to the several temperatures. The mean Q_{10} of all determinations (17) is 1.56, a value that approximates closely the temperature coefficient of conduction velocity in the same temperature range (Gasser, 1931). The coefficients based on the lower ranges of temperature do not seem to be any larger than those based on higher temperatures. In this respect the summation interval apparently differs from other manifestations of nerve response, namely, action potential, absolutely and relatively refractory phases and conduction rate. Keith Lucas (1910) states that the Q_{10} of the summation interval of muscle is 1.3.

4. *Effect of polarization.* The summation interval that develops in response to a submaximal induction shock is materially longer when the shock is delivered, after the initial transient in irritability, at the cathode

of a constant current of less than rheobasic voltage than the summation interval produced by the same shock at the same relative strength with respect to threshold delivered to the same nerve when unpolarized. Figure 4 is a case in point. Here the summation interval developing in the normal nerve lasted 0.75σ, in the nerve, when polarized, 1.35σ (13.5°C.).

The electrical artifact and the summation interval. The evidence derived through the use of shocks of measured duration and strength, and through modification of the nerve by temperature and polarization all indicates that the summation interval outlasts, and is not primarily determined by the duration of, the sensitizing shock provided the latter be sufficiently brief. But the electrical equilibrium in nerve as measured by the electrical artifact or escape is disturbed by a shock through a period that usually outlasts considerably the shock duration. The factors that enter into the

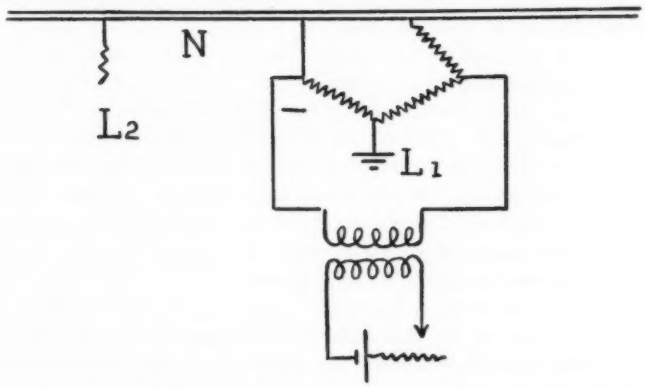

Fig. 5. (1/10/31) Balanced circuit for diphasic lead from the stimulating cathode. *N*, nerve; L_1, ground lead; L_2, grid lead.

make-up of the escape (Bishop, Erlanger and Gasser, 1926; Bishop, 1929) need not concern us here, but only the question of a possible relation between escape duration and summation interval. That there is no relation is easily demonstrable; for it is possible to give the escape almost any duration by varying the balance in the circuit with respect to the ground, without effecting the duration of the summation interval in the least. By adjusting this balance one can make escape and summation interval coterminal. Then it can be shown that this relation is entirely fortuitous by changing the temperature of the nerve; this affects the escape almost imperceptibly, whereas the summation interval changes in accordance with its Q_{10} of about 1.6.

As there actually is a summation interval outlasting the applied potential the question arises, *has the summation interval an electrical sign?* The sign

that one would expect to find, if there were one, would be a transient unpropagated potential developing at the site of stimulation and lasting as long as the summation interval. To put the question to the test the circuit shown in figure 5 was used. By the method of balancing previously used in this laboratory (Bishop, Erlanger and Gasser, 1926) the shock escape was reduced to a minimum and a record was made of the potential developing in the nerve at the point stimulated. Both of the leads into the oscillograph were from intact nerve so that potentials propagated in the manner of action potentials would record diphasically, unpropagated potentials, monophasically. In a perfectly satisfactory experiment with four-panel amplification no local potential was found that was as long as the summation interval until a diphasic effect appeared, and this had the characteristics of, and, undoubtedly was, the action potential. We have not found it possible, therefore, to correlate any electrical phenomenon in nerve with the summation interval.

Relativity of sensitizing and testing shocks. The sensitizing shock is below the threshold of all of the fibers, whereas the testing shock, since it is submaximal, is below threshold of some, the less irritable, of the fibers. The submaximal shock, therefore, should sensitize the fibers not responding to it. That it does was disclosed when inadvertently the testing shock was carried into the sensitizing shock from before. As the former approached the latter the action potential increased in height when the interval became less than the summation interval. Analysis of the records showed that then the action potential actually became double, consisting of a small action potential determined by the "testing" shock and a higher action potential later than the latter by an interval equal to the interval between the two shocks, and undoubtedly the result of stimulation of fibers sensitized by the "testing" shock.

The *configuration of the summation curve* throws little, if any, light on the nature of the processes that enhance the irritability. As has been pointed out, the summation interval unquestionably is made up of two parts, namely, the period during which the shock potentials overlap, and the succeeding period when they do not; there is no obvious discontinuity on the irritability curves at the point where the shock potential terminates.

The phase of depression: The transition. The summation interval has passed into the *phase of depression*, as we shall designate the period of depressed irritability, without any obvious discontinuity, excepting one experiment. In this case, figure 4, the summation was protracted by cathodal polarization and lasted 1.35σ (13.5°C.). The figure shows clearly a discontinuity between the summation and depression phases. The fact that this feature is seen in a protracted curve suggests the possibility that it is constantly present, but masked by the speed of the fall in irritability; but the number of experiments done under these conditions is too small to

justify any such generalization. The discontinuity is not seen when the process is prolonged by cold.

Configuration and duration of the phase of depression. The depression reaches its maximum relatively rapidly and recovery then takes place more slowly, irritability approaching normal asymptotically. The falling and rising phases are affected by strength of shock and by temperature. The depth of this negative phase, however, varies very decidedly from nerve to nerve under conditions to all appearances comparable. Occasionally it is so poorly developed that it can scarcely be detected.

Increasing the *strength of shock* increases the depth of the curve, moves the trough slightly later and seems to prolong recovery (see figs. 3 and 6). The point where recovery is complete, the termination of the asymptotic return to the normal level, is difficult to locate precisely; and anything

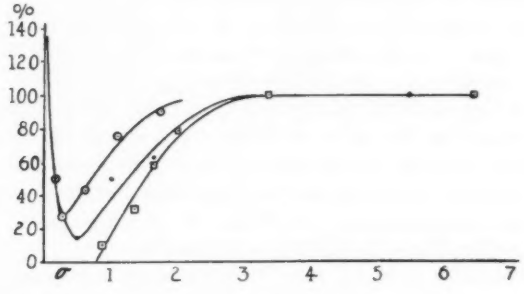

Fig. 6. (2/12/30) Irritability responses, 26.0°C.
Dots = Sensitizing shock just below threshold, coil at 37.8.
Circles = Sensitizing shock considerably below threshold, coil at 43.
Squares = Curve of relative refractoriness.
All plotted in per cent of a very much submaximal normal action potential.

delaying or deepening the down stroke, by steepening the recovery curve, produces the impression of a lengthening of the recovery time. For this reason the relation of shock strength to the duration of the phase of depression is guardedly stated. The changes induced by increasing the strength of the sensitizing shock have their limit, of course, where the shock reaches threshold value, for then the nerve fires off and the picture is changed by the intrusion of the refractory states, absolute and relative.

The relation of the phase of depression to the relatively refractory phase: We early became interested in the possibility of a relation between the return of irritability to the normal level after depression determined, *a*, by a sensitizing shock, and *b*, by a complete nerve response. The methods of obtaining data for the plotting of the relatively refractory phase have been described in another place (Gasser and Erlanger, 1930); for the most

part use has been made here of the one in which the second action potential is larger than the first; the two shocks, however, were delivered through the same pair of electrodes. A set of curves illustrating the results regularly obtained is shown in figure 6. The temperature was 26°C. Here the amplitude is expressed in per cent of the normal height of the action potential, which, for all three curves, was roughly the same absolutely, namely, between 3 and 4 cm. Since the amplification was about 100,000 "normal height" was a very small fraction of maximal height. This is mentioned lest the impression be given by the group of curves that the negative phase has an amplitude range approaching that subtended by the curve of the relatively refractory phase as ordinarily made, namely, from 0 to maximum. This, of course, is not the case; a subthreshold sensitizing shock would reduce very slightly the amplitude of an action potential elicited by a just maximal testing shock.

The curves of figure 6 are suggestive of two relations which may be of some significance. As the strength of the sensitizing shock increases the lowest point on the curve moves later in such a way as to give the impression that it is approaching the duration of the absolutely refractory phase (the lowest point on the curve of relative refractoriness) as a limit. A similar tendency is seen in a number of the experiments; there have been exceptions, however, in which the low point has occupied a position that was later than the position of the return of responsiveness. It is quite possible, therefore, that this relation is fortuitous.

The other relation is much more definite; with increasing strength of sensitizing shock the phase of depression approaches the duration of relative refractoriness as a limit. With relatively short induction shocks and under ordinary conditions this has regularly been the case (see figs. 3, 6 and 9).

Effect of temperature on the relation between the phase of depression and the relatively refractory phase. To ascertain whether this is a fixed relation it has been followed in nerve exposed to wide temperature changes. In summer frogs living at temperatures of 26 to 30°C. (systematic tests have not been made at other seasons of the year) it holds perfectly between 15° and 28°C., a range that alters the duration of the phase of depression (and of the relatively refractory phase) as much as 300 per cent. At temperatures below about 12° to 15°C. this relation breaks down; at that point an enormous prolongation of the relatively refractory phase begins to develop and it then terminates much later than the depression interval. The temperature at which this disparity develops corresponds with that (12° to 13°C.) at which the supernormal phase and the after-potential disappear (Gasser and Erlanger, 1930) and which, therefore, may be regarded as critical of a number of nerve reactions. In the light of some recent observations on the effect of temperature on nerve (Gasser, 1931) our results

may be interpreted as meaning that the two phenomena under consideration are determined by the same mechanism, one that is influenced by common factors at temperatures between 15° and 28°C., but at lower temperatures by additional factors which have the effect of prolonging the recovery of nerve that has been excited. In other words it is felt that emphasis should be laid rather on the correspondence that obtains at ordinary temperatures than on the dissimilarity that develops at, considering the season, abnormally low temperatures.

Irritability changes at the anode. For the interpretation of the foregoing results it seemed desirable to obtain comparable data on the irritability changes that develop at the *anode* of a subthreshold induction shock. For

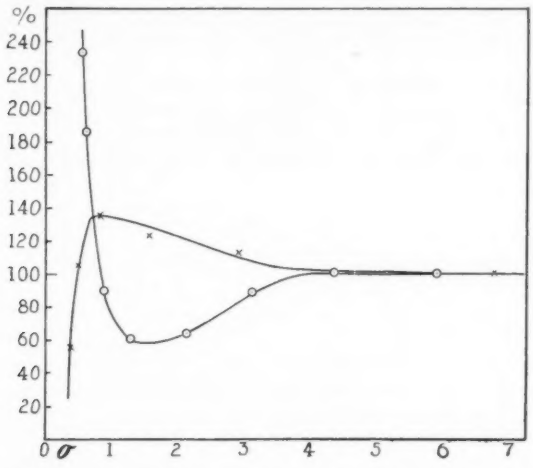

Fig. 7. (6/14/30) The irritability responses to a cathodal (circles) and an anodal (crosses) sensitizing shock, testing shocks slightly superthreshold. 25.0°C. Ordinates, amplitude of action potential in per cent of normal.

these experiments the direction of the primary current of the sensitizing coil is reversed so that the proximal terminal on the nerve becomes positive on the break instead of negative. When this is done the irritability picture, as determined in the usual manner by the testing shock, is completely inverted (see figs. 7 and 9); the curve begins below the normal level, instead of above, and this phase is then followed by a long period of enhanced irritability ending asymptotically, in the place of the phase of depression. Possibly there are slight quantitative differences between the corresponding phases of the responses to the anodal and cathodal shocks. Following the anodal shock the initial negative phase definitely is briefer than the summation interval which follows a cathodal shock, and the crest of the

subsequent positive phase is earlier than the trough of the cathodal negative phase. The impression also is obtained that the positive phase

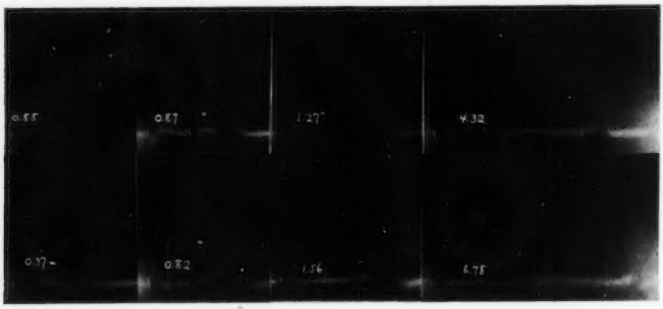

Fig. 8. (6/14/30) A few of the records supplying the data for figure 7. The shock escapes give the time relation of the testing to the sensitizing shocks. Their separation in sigmas is given by the numerals. The upper series of action potentials was obtained with sensitizing shock cathodal; the lower, with sensitizing shock anodal. $\times\frac{1}{2}$.

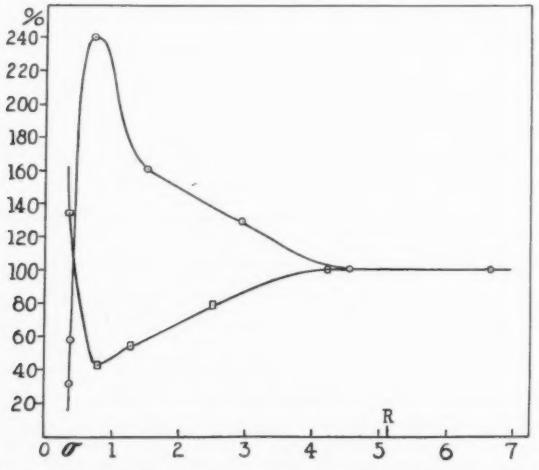

Fig. 9. (6/12/30) The irritability responses to a cathodal (squares) and an anodal (circles) sensitizing shock, testing shocks slightly superthreshold. 24.2°C. *R* marks the end of the relatively refractory phase. Ordinates, amplitude of action potential in per cent of normal.

produced by the anodal shock falls off more rapidly initially but reaches the normal level somewhat later than the negative phase following a cathodal shock.

Figure 8 consists of a few of the records upon which the curves of figure 7 are based. These are contact prints of the picture on the screen of the tube and are much less sharp, owing to irradiation, than the pictures the eye sees.

DISCUSSION. Whatever the significance of these differences may be, they do not stand in the way of the obvious deduction that the phase of depression produced by a subthreshold cathodal induction shock and the positive phase of the irritability change produced by an anodal shock are manifestations of the well-known postcathodal depression and postanodal enhancement of irritability, respectively. If it be granted that the phase of depression at the cathode of a subthreshold induction shock is a postcathodal depression then, since in its full development and at all reasonable temperatures it has the same duration as the relatively refractory phase,

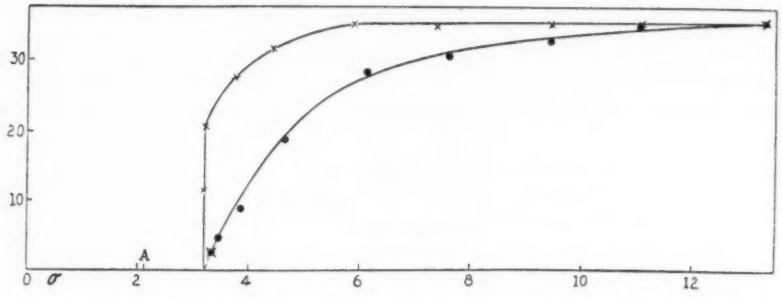

Fig. 10. (2/24/31) The relatively refractory phase (dots) and its modification (crosses) by an anodal shock so placed in the absolutely refractory phase (at *A*) that the postanodal phase of enhanced irritability falls in the period of relative refractoriness. 13.0°C. Ordinates, amplitude of action potential in millimeters. Abscissae, sigmas, first shock at zero.

the conclusion seems to be warranted that the latter also is a period of postcathodal depression left in this case by the nerve's action potential. This conclusion is very strongly supported by the observation noted by Kato and collaborators (1929) that an action potential that is stopped by a locally persisting relative refractoriness increases and maintains the refractoriness. It seems reasonable to infer that under these circumstances the blocked action potential acts as if it were a subthreshold induction shock and, like the latter, leaves behind the characteristic postcathodal depression. If the action potential can do this when it fails to stimulate there is every reason for believing that it would act similarly on nerve that is responding and leave in its trail a state of postcathodal depression which is, or at least contributes to, the relatively refractory phase. On this basis the Wedenski block, which is attributed by Kato to a repetition of

his "after-effects" at a rate so rapid that refractoriness persists, must be regarded as block due to maintained postcathodal depression.

Modification of the relatively refractory phase by postanodal enhancement. If the relatively refractory phase is a state of postcathodal depression of irritability it should be possible to abolish it by a properly placed state of postanodal enhancement of irritability. A few experiments have shown that in very large measure this can be done. Figure 10 presents the results of such an experiment. It was performed on a nerve of a winter frog at 13°C. The record of the action potential was obtained directly from the stimulated point on the nerve (see fig. 11). At zero time the nerve was

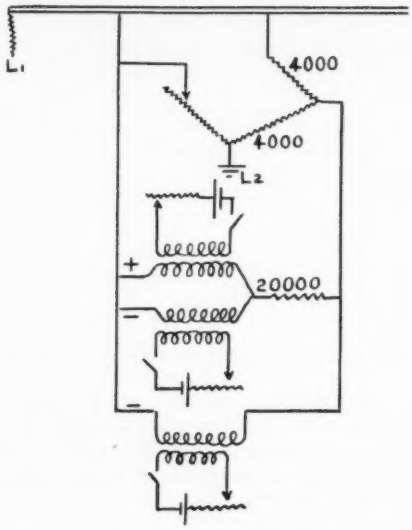

Fig. 11. (2/24/31) Circuit for the modification of the relatively refractory phase by an anodal shock. Lead (L_2) from the point stimulated. See text.

stimulated with a supermaximal cathodal break induction shock and data for the plotting of the relatively refractory phase were obtained by ascertaining the height of the action potential that resulted when, at various intervals thereafter, the nerve was stimulated through the same electrodes with a cathodal supermaximal break shock from another secondary coil. The curve obtained is marked by dots in figure 10. Then, without changing any other of the conditions, this procedure was repeated, but now a break shock from a third secondary coil was added, this one, however, with its anode at the lead. This shock was made to fall (at A) in the absolutely refractory phase of the action potential produced by the activating (first)

shock. It, also, was supermaximal but did not stimulate. The insertion of this anodal shock alters very decidedly the configuration of the relatively refractory phase (curve marked by crosses). The nerve regains more than half of its normal irritability at the instant the absolutely refractory phase ends, and becomes normal less than 3σ thereafter, whereas, without the anodal shock, the attainment of normal irritability required more than 12σ. Obviously the anodal shock markedly reduces the relatively refractory phase. It remains for further experimentation to determine whether complete abolition of relative refractoriness can be accomplished by this method of procedure. As the matter stands the possibility remains that there is a component of the relatively refractory phase that owes its origin to some other state than the postcathodal depression left by the action potential.

SUMMARY

The changes in irritability incident to the stimulation of nerve with a subthreshold induction shock are followed, using as an index the action potential of the nerve, observed, after amplification, on the cathode ray oscillograph.

The duration of the first, or positive, phase, or summation interval is not affected by shock duration when the latter is sufficiently brief; but the duration of the summation interval is influenced by the strength of a subthreshold shock of any given duration. Duration also varies with the temperature, and is longer in cathodally polarized than in normal nerve.

It is definitely demonstrated that the summation interval can outlast the applied shock potential and varies independently of the electrical artifacts.

It has no recognizable electrical sign.

The second or depression phase returns to the normal level asymptotically in a period that seems to increase with the strength of the sensitizing shock, but at temperatures between 28° and 15°C. approaches in duration the relatively refractory phase as a limit.

When the subthreshold sensitizing induction shock is anodal instead of cathodal the entire irritability picture is inverted, the terminal phase being one of enhanced instead of depressed irritability. The second phase following a cathodal shock, therefore, is regarded as a state of postcathodal depression.

Since the depression interval left by a just subthreshold cathodal shock is coterminal with the relatively refractory phase, the latter also is regarded as a state of postcathodal depression left in this case by the nerve's action potential. This interpretation is supported by the observation that the relatively refractory phase can be almost abolished by a properly placed anodal shock.

The "after-effect" of Kato that is left by a blocked nerve impulse, the repetition of which is believed by him to produce Wedenski blocking, therefore is to be regarded as a state of postcathodal depression and the Wedenski block as a state of maintained postcathodal depression.

BIBLIOGRAPHY

BISHOP. 1929. This Journal, lxxxix, 618.
BISHOP, ERLANGER AND GASSER. 1926. This Journal, lxxviii, 592.
BROEMSER. 1921. Zeitschr. f. Biol., lxxiii, 19.
ERLANGER, BISHOP AND GASSER. 1926. This Journal, lxviii, 527.
GASSER. 1931. This Journal, xcvii, 254.
GASSER AND ERLANGER. 1930. This Journal, xciv, 247.
GILDEMEISTER. 1908. Pflüger's Arch., cxxiv, 447.
KATO AND ASSOCIATES. 1929. This Journal, lxxxix, 692.
LEVINSOHN. 1910. Pflüger's Arch., cxxxiii, 267.
LUCAS, K. 1910. Journ. Physiol., xxxix, 461.
WEDENSKI. 1883. Arch. f. Physiol., 313.

THE IRRITABILITY CHANGES IN NERVE IN RESPONSE TO SUBTHRESHOLD CONSTANT CURRENTS, AND RELATED PHENOMENA[1]

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The investigation reported in the preceding paper, dealing with the irritability changes initiated in nerve by subthreshold induction shocks, led to the conclusions, among others, that the summation interval may outlast the potential that develops in the inductorium and that the subsequent phase of depression is to be regarded as a postcathodal depression of irritability. In the hope of obtaining further light on the nature of these two reactions to shocks a similar study was undertaken of the alteration in irritability occurring in response to subthreshold constant currents of known duration and form. The study includes an analysis of the changes in irritability incident to the make and break and of the response to shocks in nerve steadily polarized as compared with normal nerve. Some attention has been given also to the irritability changes developed by currents growing at less than the liminal rate.

The literature dealing with the irritability changes developing in association with constant currents is so vast that to review it here would be quite impossible. It must suffice to refer the reader to the very good account given by Gotch in Schafer's *Textbook of Physiology* (1900). The particular phases that concern this investigation are summed up there as follows: With closure of a constant current the irritability at the cathode rises instantaneously, increases rapidly to a maximum and then subsides quickly if the closure is sufficiently long; when the current is opened the fall of excitability is established instantaneously and subsides rapidly. Recently Bishop (1928a) has determined the configuration of the increase and decline in irritability at the cathode that accompany the make of a subthreshold constant current. His findings and inferences can best be presented later on.

GENERAL METHOD. In the present research the routine procedure, from which there have been many departures, has been to sensitize the

[1] The results on which this paper is based were presented at the meeting of the American Physiological Society, April 10, 1931 (This Journal, 1931, xcvii, 519).

nerve with a subthreshold constant current, and determine the irritability changes developing at the cathode, and in a few experiments at the anode, by means of a testing induction shock, using the criteria described in the preceding paper.

A number of circuits have been employed during the course of the work, but most use has been made of the one shown in figure 1. All of the resistances were nonreacting. The opening of the short-circuiting switch K_1 and of the series switch K_2 determine the time the nerve is exposed to the constant current. By means of the potentiometer it can be given a known percentage of threshold value. In other circuits, used to some extent, the short circuit shunted not only the sensitizing constant current but also

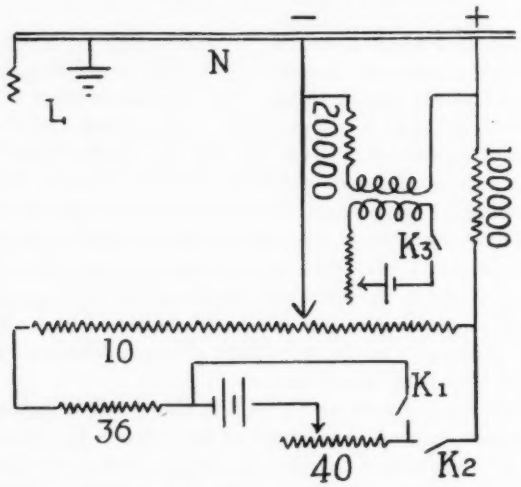

Fig. 1. Diagram of circuit for testing the irritability of nerve at the locus of polarization. *N*, nerve; *L*, leads.

the testing secondary circuit; and in still others the period of constant current flow was determined simply by switches in series. By shifting switch K_3 around the circumference of the wheel of the synchronizer, the testing shock can be placed wherever desired with respect to this current. During any series of observations the rotating synchronizer was run continuously, the cycles repeating themselves at the rate of about one per second. By a "long" constant current is meant one lasting 30 to 40σ, or 3 to 4 per cent of a cycle. The duration of "short" constant currents is always given. In other respects the methods were in general similar to those used in the preceding study.

Configuration of the constant current. It is seen in figure 1 that the cur-

rent flows not only through the nerve but also through the secondary coil. Since self-inductance will delay the building up of the current through the coil it is to be expected that with the make of the constant current the flow through the nerve will momentarily be above its final level; that with the break it will momentarily fall below the zero level. By directly recording with the Braun tube the potential as it is delivered to the nerve by the circuit shown in figure 1 this predicted transient can readily be demonstrated. The constant current in such an experiment reached its maximum in about 0.02σ and was back at its steady level in about 0.07σ. The transient potential exceeded the steady potential by about 25 per cent.

Interaction of constant and induced currents. It is well known that a testing shock must be given an appreciable strength in order to stimulate a nerve that is about to respond to a constant current of rheobasic voltage. It seems natural to assume that a nerve under these circumstances would require to complete the stimulation only an infinitesimally small additional polarization and that consequently the shock strength needed to stimulate then measures, not the irritability of the nerve, but rather an opposing balance of the constant current flow and the induced current flow through the secondary coil in a circuit such as we use, and, therefore (see Bishop, 1928b), can be entirely disregarded. Since the magnitude of this effect must be taken into consideration in the interpretation of irritability measurements in polarized nerve we have attempted to ascertain how significant it is. One method has been to directly register on the cathode ray oscillograph the induction shock obtained through the circuit of figure 1 under conditions exactly comparable with those obtaining in our routine procedure. The pictures demonstrate that the shock and the constant current sum perfectly within the limit of error of the method (about 10 per cent). This result has been verified by the ballistic method. It may be stated, therefore, that, within these limits, the shock is changed neither in regard to height nor in regard to configuration by the introduction of the polarizing current. Under the circumstances, it would seem that, as a measure of irritability, the strength of the primary current has the same significance in polarized that it has in normal nerve.

RESULTS. *The initial alteration in irritability at the cathode.* When the irritability, as measured by the amplitude or constant height methods, is determined by moving the testing shock into the constant current from before, it begins to increase before the testing shock and constant current overlap. This is due to stimulation by the subthreshold constant current of fibers that escape stimulation by the submaximal testing shock but are in the phase of the summation interval set up by the latter. Mounting rapidly, but with decreasing speed as the testing shock is moved later, the irritability curve soon reaches a rounded ascending, or level plateau (see

figs. 2 and 3). From the end of this plateau the irritability drops off sharply and, in a consistent curve that is definitely concave upwards from the start practically in every case, it approaches asymptotically a level, usually steady, that persists to the break of the constant current. This *maintained* irritability, as we shall designate it, occasionally rises for a time, or still more rarely falls. To long, just threshold, constant currents the nerve responds at or shortly after the end of the plateau; the response,

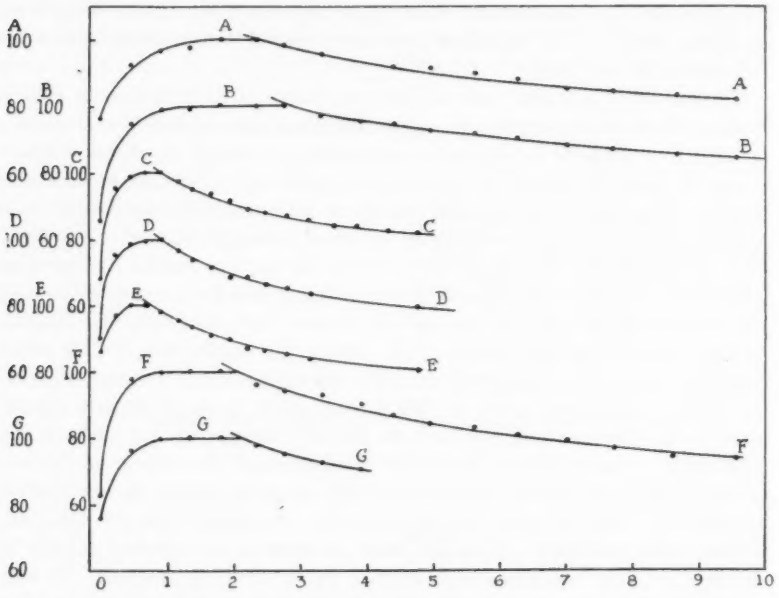

Fig. 2. (3/3/31) The effect of temperature on the "irritability" responses initiated by the make of a constant current, 90 per cent of rheobase. Constant height method. Successive determinations on the same preparation; *A* and *B* at 12.0°C., *C*, *D* and *E* at 24.2°C., *F* and *G* at 12.2°C. Ordinates, per cent of maximum irritability in each case. Time in sigmas, the make of the constant current being at zero. Normal levels not shown.

therefore, may develop some time after the irritability, as measured by an induction shock, has attained its maximum level.

In an effort to ascertain the factors that contribute to these initial irritability changes we have determined the configuration of the curves as affected by changing conditions, such as temperature and the strength of the polarizing current.

The effect of temperature. The time occupied by the phases of the curve

is markedly influenced by temperature, but these temperature effects are not entirely constant. To give an example, in the experiment furnishing the data for figure 2 the interval extending from the start of constant current to the end of the plateau measures in sigmas in the successive determinations, 2.35 and 2.77 at 12°C.; 0.92, 0.92 and 0.68 at 24°C.; and 2.00 and 2.10, again, at 12°C. In other experiments the fluctuations have been wider, to such a degree, even, as occasionally to yield negative temperature coefficients.

In cold (12°C.) nerve the plateau is a very definite and striking feature of the irritability curve, but in warm nerve (room temperature) the rising

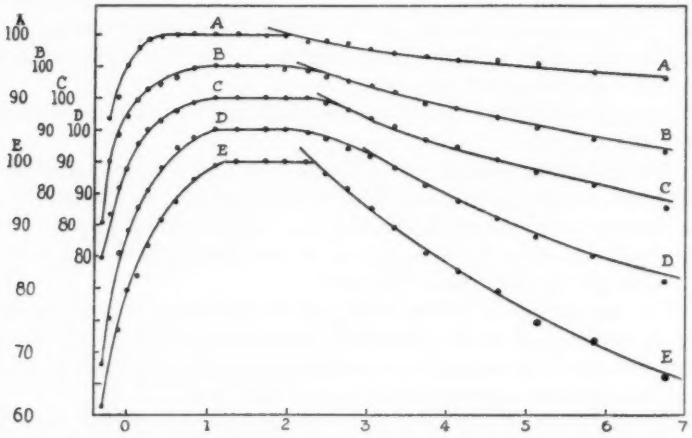

Fig. 3. (3/19/31) Effect of strength of polarizing current on the irritability response initiated by the make of a constant current. Constant height method. Successive determinations on one preparation; *A*, constant current 50 per cent, *B*, 75 per cent, *C*, 90 per cent, *D* 90 per cent (three panels), *E*, 100 per cent of rheobase, determined, with the exception of *D*, at four panel amplification. Ordinates, per cent of maximum irritability in each case. Time in sigmas with make of constant current at zero. Normal levels not shown.

phase occasionally appears to be cut short by the falling phase before a plateau stage is reached. Possibly the plateau is present in such cases but at times is missed because, being brief, the number of points is insufficient to outline it. Temperature does not affect materially the depth of the decline after the plateau, but has a marked effect on its duration.

The effect of polarization strength. The configuration of the initial irritability changes as affected by the strength of the polarizing current is seen in figure 3. In this experiment five subthreshold constant current strengths were used. The preciseness of the determination of threshold value depends to a considerable extent upon the amplification used. What seems

to be threshold with three-panel amplification is found to be considerably above threshold strength when the fourth panel is added. This accounts for the difference between the curve *C* (90 per cent, four panels) and curve *D* (90 per cent, three panels), and justifies the statement that the figure depicts data derived from observations at five strengths of polarizing current. In order to obtain the range of primary current needed to test irritability with the resistances at the moment available for this experiment it was necessary to adjust the position of the secondary coil with each change in constant current strength. As the coil readings were not noted it is impossible to so plot the data as to bring out the irritability relations of the curves to each other. To illustrate this relation other experiments will be used. Each curve shows the irritability range expressed in per cent of the irritability obtaining at its plateau. The curves present certain features which may be of interest.

1. Attention is again called to the plateau and to the abrupt beginning of the falling phase.

2. The irritability range between the plateau and the maintained levels (in this experiment observations were discontinued before the curves had quite flattened out; therefore, the height of the maintained levels can be ascertained only roughly) increases at an accelerating rate with increase in the strength of the constant current.

3. The time occupied by the rising phase definitely increases with increase in the strength of the polarizing current; in the case of the weakest current here used (50 per cent of threshold) the plateau is reached in 0.80σ and of the strongest current (100 per cent) in 1.36σ.

4. The plateau seems to break later the stronger the current; therefore, the plateau stage seems to maintain its duration but to shift into later positions as the current strength increases.

Other experiments show that the irritability obtaining at the time of the plateau increases at an accelerating rate with increase in the strength of the polarizing current up to the threshold level. In this same range the maintained irritability likewise increases, usually at an accelerating rate, also, but more slowly, so that an increasing separation of the irritability at the two levels develops as the constant current strength increases. Figure 4 gives the results of a typical experiment (27°C.) in which the curve of maintained irritability (circles) accelerates slightly, at least, at first. The points plotted are each averages of several determinations that agree fairly well among themselves. Both curves should, of course, pass through 23, the irritability of unpolarized nerve. The failure of the curve of the plateau levels, as recorded, (dots, upper), to do so is regarded as an expression of experimental error; it could be accounted for by a 2 per cent error in the reading of the scale. A more correct conception of the relation of plateau to maintained irritabilities, we are sure, is obtained by

arbitrarily dropping the upper curve so as to make it pass through 23. It is then seen (dots, lower) that at 5 per cent of polarization plateau and maintained irritabilities are practically alike; that even at 50 per cent of rheobasic polarization they are apart less than 3 per cent of the normal-to-rheobasic irritability range. The separation then increases rapidly so that at rheobasic polarization the height of the plateau above the maintained level amounts to about a third of the normal-to-rheobasic range in irrita-

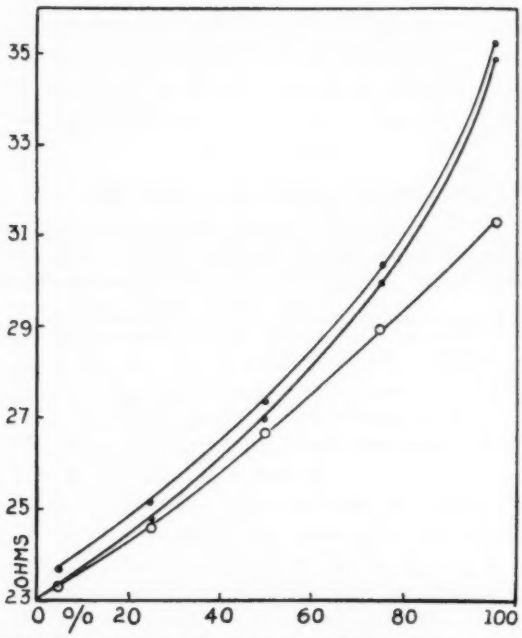

Fig. 4. (6/12/31) The irritability obtaining during the plateau and maintained stages of response to polarization, the latter about 20σ after the constant current make, as affected by polarization strength. 27.0°C. Ordinates, the primary resistance of testing circuit in ohms; abscissae, the constant current strength in per cent of rheobase. Dots, plateau irritability, the upper as recorded, the lower as corrected; circles, the maintained irritability.

bility. Indeed, if the induction shock method of measuring irritability were perfect the upper curve at this point presumably would be at infinity; for the constant current by itself would suffice to produce the response.

The irritability at the plateau cannot be followed beyond that determined by strengths of constant current exceeding threshold, for then the nerve responds. Observation of the maintained irritability, however, is not thus limited; it is only necessary, in order to minimize the conse-

quences of the nerve's response, to allow the current to act until the transient relative refractoriness has entirely disappeared before making the

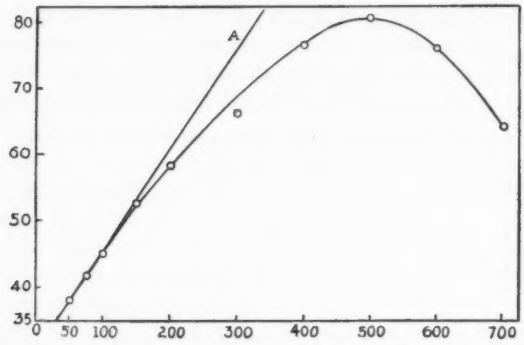

Fig. 5. (6/30/31) The irritability obtaining during the maintained stage of polarization, as affected by polarization strength. 28.8°C. Ordinates, reciprocals of primary amperage; abscissae, polarization strength in per cent of rheobase.

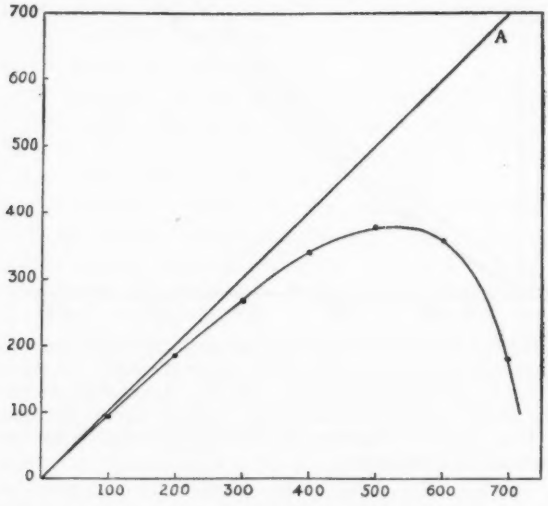

Fig. 6. Construction of a curve resembling that given by the relation between irritability during the maintained level of polarization and the strength of the polarizing current. See text.

readings. The time the current has acted when these tests are made must, of course, be the same in comparable observations. Figure 5 presents the data from an experiment in which the irritability during the maintained

stage was followed through a range of polarization strength exceeding the rheobasic (= 100 per cent) seven times. In this case the irritability rises at a retarding rate from the start so that finally (at about 500 per cent polarization) the curve bends toward the X axis.

This curve suggested the construction shown in figure 6. The ordinates of this graph represent irritability in arbitrary units, with zero at the normal, unpolarized level. The abscissae represent strength of polarization, rheobasic being 100. The line OA bisects the angle at the origin. From the intersection of every hundredth abscissa and OA, beginning with zero, a point is laid off downward as follows: from zero, 3.75 ordinate units, from 100, 7.5, from 200, 15, from 400, 30, and so on. We are told that the expression for this graph is

$$y = x - be^{kx}$$

We are more interested, however, in the close resemblance this graph bears to the curve (fig. 5) derived from experimental data. They both rise with decrementing rate to a crest, attained when polarization acquires a value of about 500 per cent. At this point both curves bend toward the X axis, descending rapidly and along similar lines.

To derive by construction curves simulating those seen more commonly (see fig. 4), in which the rise of the maintained irritability at first is at an accelerating rate, it would be necessary to substitute for the line OA a rising curve accelerating during the subrheobasic period, at least. The equation then would contain two exponential expressions, one, as at present, negative, the other positive and replacing x.

Discussion. In considering the factors that determine the irritability changes occurring in response to the make of a subthreshold constant current it is necessary first to ascertain what rôle, if any, is played by the initial transient resulting from the presence of the secondary coil in the circuit. It has been seen that this transient lasts only 0.07σ, whereas the initial irritability changes may occupy over 2.0σ. For this reason, and on the basis of the experiments on temperature, the transient seems definitely to be ruled out as a factor; for while temperature affects markedly the duration of the initial irritability changes, its effect on the duration of the electrical transient of the activating circuit is inappreciable.

It has been stated that Bishop (1928a) studied the configurations of the increase and decline in irritability determined at the cathode by the make of a constant current. The stimulating effect of the current, he found, follows a curve similar to that of the counter E.M.F. of two condenser systems in series. However, the two irritability curves he reproduces differ from ours by the absence of a plateau and in having a descent to the maintained level that begins gradually with an initial upward convexity instead of abruptly and with a consistent upward concavity.

The next question to consider, therefore, is whether the configuration of the irritability curves derived from our data could be determined by two independent but concurrent processes, and if so, what might be their nature. It has been seen that the curve representing the relation, during the maintained level, between irritability and constant current strength includes two expressions both of which may be exponential, one positive and the other negative. This very naturally suggests the thought that the irritability picture is the result of two processes beginning simultaneously, one increasing irritability, the other decreasing it. Therefore, attempts have been made to find two smooth curves starting in opposite directions

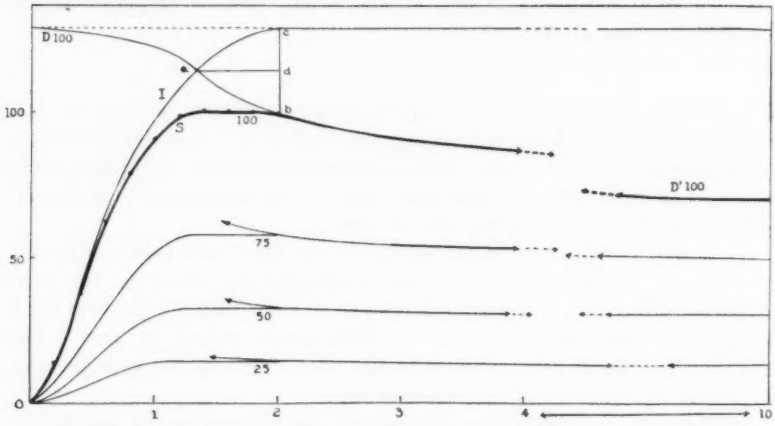

Fig. 7. Construction to show that the irritability changes developing at the cathode in association with the make of a constant current could be determined by two processes, one quickly increasing irritability, the other decreasing it more slowly, both beginning simultaneously. Ordinates, irritability, zero being normal and 100, the maximum obtained at rheobasic voltage. Abscissae, duration of current flow in sigmas, zero corresponding with the make. The constructions for the results obtained in response to weaker polarizations, 75, 50 and 25 per cent of rheobase, also are shown. See text.

from zero which, by algebraic addition, would yield the irritability curves derived experimentally. The premises have been as follows. The curves of figures 5 and 6 portray the relation of irritability to strength of current. But, if instead of strength, the time of action of a current of constant strength were the variable plotted on the abscissa, it is clear that the two curves arbitrarily chosen, instead of bending toward the X axis, would approach limits which would in all probability parallel the X axis.

With these considerations in mind the construction seen in figure 7 was evolved. For this diagram the plateau and maintained irritabilities used

are those of figure 4, the durations, approximately those of figure 3. The curve I represents the action on the process that enhances irritability, DD' the action on the process that depresses irritability. To facilitate subtraction of D from I the start of the former is placed not at zero, where it belongs, but on the level of the upper asymptote of I. Subtraction will yield a plateau only if and where the inclinations of the two curves are equal and opposite. To approximate this relation through the desired stretch it is necessary to give curve DD' an inflection such that the figures *acd* and *abd* become as nearly as possible superimposable. Perfect superimposability seems to be impossible of attainment without the introduction of a sharp discontinuity in D at b. Addition of D and I gives the curve SD'. This fails exactly to match the irritability curves in one respect; between the plateau and its upwardly concave termination there is a short stretch slightly convex upwards. However, this deviation from experiment is so slight that one would be inclined to assign it little weight were it not for the practically regular absence in the irritability curves of any tendency to decline prior to the discontinuity at the end of the plateau by whatever method derived.

The alternative view is that the irritability changes developing at the cathode with the make of the constant current are the result of *two successive processes*, one extending to the end of the plateau, the other thence to the beginning of the maintained level. To attempt the formulation of a working hypothesis based on this conception one might suggest that the first stretch depicts preparation of the irritable mechanism to respond, whatever that may connote, and the second stage a recovery to the steady state determined by the polarization in the event of failure to respond. This hypothesis is not wholly lacking in support. For instance, the time to the end of the plateau often closely approximates the duration of the absolutely refractory phase at the same temperatures. This relation, though, we believe to be entirely fortuitous, particularly because of the variability of the relation between the time to the end of the plateau and temperature, to which reference was made above.

But irrespective of whether the decline in irritability begins at zero time and is masked up to the end of the plateau by another process that increases irritability, or begins later at the end of the plateau, it seems necessary to regard it as a manifestation of so-called cathodal depression. If it be cathodal depression starting with the make of the polarizing current it will be necessary to assume that the process determining cathodal depression and the process determining cathodal enhancement each increases at the same proportional rate as the polarizing current after as well as before the nerve responds. This is a necessary consequence of the absence from curves (fig. 5) portraying the relation between the irritability at the maintained level and the strength of the polarizing current of a discontinuity at the

point where the constant current strength exceeds the rheobase; at that place the nerve passes through the absolutely and relatively refractory phases without manifesting any disturbance in the rate of increase of the maintained irritability. The concept that cathodal depression manifests itself only after the completion of the plateau stage does not seem reasonable, and affords no clue to a method of graphic representation.

That the irritability level of nerve during polarization is determined by two processes, one acting rapidly and enhancing irritability, the other more slowly and reducing irritability, the latter becoming relatively more and more effective with increase in polarization strength, is supported by the drop in irritability below its normal value that occurs when the current is broken; for this postcathodal depression, to be considered later, can be regarded as evidence of a preëxisting but masked alteration of the polarized nerve in the direction of depression which is uncovered and disappears slowly with the cessation of polarization. Werigo (1883), from the beginning, regarded cathodal depression as a constant phenomenon, developing so quickly with the make of a strong current as to mask the initial rise in irritability, with weak currents more slowly, many minutes (20 to 30) then being required for depression to manifest itself, depression in the Werigo sense consisting of a lowering of irritability below the normal level. If the decline from the irritability maximum following the make of a subthreshold constant current is a sign of a depressing action of cathodal polarization, Werigo's view regarding the universality of cathodal depression could be amended so as to indicate that the manifestations of depression develop as quickly with weak as with strong currents.

THE PRETERMINAL PERIOD AND RELATED PHENOMENA. When the submaximal testing shock delivered as usual at the cathode is carried through the maintained level toward the break of a long subthreshold constant current the action potential begins to diminish in height before the shock reaches the end of the constant current. The interval between the shock position at which this apparent decline in irritability begins and the break of the constant current, designated, for the sake of convenience, the *preterminal period*, lasts 0.3 to 0.7σ, possibly longer, depending upon conditions to be considered.

The preterminal phase can not possibly be regarded as an effect of the break of the constant current upon irritability, unless perchance it were the expression of a defective breaking mechanism causing the polarizing current to fall off before the actual break; and we have been able to satisfy ourselves that this is not the case. Direct observation by means of the cathode ray oscillograph shows that the constant current switch is operating perfectly, producing a clean, sharp break. Furthermore, altering the temperature of the nerve alters very materially the duration of the preterminal period; it has a Q_{10} of about 1.6 in the observed temperature range.

That the behavior of the constant current switch is not the cause of the phenomenon is indicated also by the observation, to be considered again below, that a similar decrease in the amplitude of an action potential determined by a testing shock during subthreshold cathodal polarization occurs when a subthreshold break shock with cathode distal (an anodal shock) is delivered through the same electrodes later than the test shock by an interval that falls within the preterminal range. The underlying cause of the preterminal fall therefore resides in the nerve, not in the switch. The possibility also has been considered that a postcathodal electrotonic effect associated with the break of the constant current, spreading down the nerve faster than the action potential travels, might reach the lead before the action potential and reduce its recorded height; but this possibility is rendered untenable by the observation that a fully developed preterminal phenomenon is seen even when the action potential is recorded at the locus on the nerve where it is polarized and stimulated, and, therefore, long before postcathodal depression develops. We are then faced with the necessity of referring the preterminal phenomenon to a nerve response dependent upon the testing induction shock.

In the preceding paper it was shown that the break shock from the air-cored coil with the usual secondary resistances is completed in less than 0.05σ. And direct cathode ray oscillograph records show that the same is true when the break shock acts through our constant current circuit. Induction shocks, as has been said, are not altered in duration or in configuration by the constant current. The preterminal period, therefore, cannot be accounted for by direct and immediate action on the nerve of the applied shock potential.

It was demonstrated in the preceding paper that the summation interval, when present, outlasts the sensitizing shock and is longer in polarized than in unpolarized nerve. Furthermore, it has been seen in the present paper that a nerve may not respond at the instant it acquires the maximum irritability that develops through the action of a constant current, irritability remaining at a steady level during the entire period of the plateau stage before the nerve responds. Such observations as these suggest the possibility that nerve fibers might respond to brief induction shocks after intervals, *latent periods*, to employ the usual term, ranging in duration up to that of the preterminal period (or even longer), and that the preterminal phenomenon develops because of differences in response times of the component fibers of the nerve.

To illustrate how, on this basis, the preterminal phase would develop we may, for the sake of simplicity, hold the submaximal testing shock late enough in the constant current to be beyond the region of the initial transient effects, and, by stages, move the break of the constant current into earlier positions, i.e., toward the testing shock temporally. Assuming a

range of latencies of say 0 to 0.5σ, it is obvious that the height of the action potential determined by the testing shock will diminish when the interval between the shock and the constant current end becomes less than 0.5; for then the process started by the shock in the fibers that have a latency of 0.5σ will be interrupted by the disappearance of the cathodal polarization. To cause those fibers to fire off now, that is to say, to bring back the action potential to its initial height under the new conditions, it will be necessary to increase the strength of the testing shock to where it will counteract the interference introduced by the break of the constant current. The constant current break, then, has the appearance of decreasing the irritability of the nerve. This effect will grow as the constant current break moves earlier through the assumed range of latencies.

Latencies. It is thus seen that for the solution of the preterminal phenomenon information is required regarding the latency of response to a brief induction shock. With this in mind, we have determined in how far the response to a submaximal cathodal break shock can be modified by another but subthreshold break shock in the opposite direction through the same electrodes. The results obtained in this way will be illustrated by the following experiment performed at a temperature of 28.2°C. The distance of conduction was 21 mm. The first shock was from an air-cored coil and probably lasted not over 0.04σ. It was given a strength that produced an action potential of about one-quarter the maximal height. The anodal shock was just below the threshold. The nerve was polarized through the same electrodes with a current 90 per cent of rheobasic strength and the anodal shock was placed after the cathodal shock at the point where it just completely annulled the response produced by the latter. Their separation then was 0.08σ. Polarization then was stopped; the strength of the first shock was increased to the point where it produced an action potential of exactly the same height as before; and the anodal shock was again given a just subthreshold strength, but the positions of the shocks were not altered. Under these new conditions there was an action potential; it had about one-third the height of the action potential given by the cathodal shock alone. The experiment, to this point, demonstrates that the minimum latency of unpolarized fibers is shorter than that of polarized fibers. The difference in the minimum latencies was not ascertained quantitatively, but the experiment was continued by moving the anodal shock later. With this the height of the action potential increased up to normal, to the height, that is to say, of the action potential determined by the cathodal shock alone. The interval between the cathodal and anodal shocks when the height became just normal was 0.22σ in the normal nerve and 0.35σ in the polarized nerve, again under comparable conditions. These times were approximately equal to the rising times of the corresponding action potentials, which were 0.29 and 0.39σ, respectively.

In other experiments it has been found that separation of the two shocks, cathodal and anodal, continues to modify the action potential configuration for some time after the separation exceeds the time to maximum of the action potential, the change then consisting of a steepening of the action potential decline which diminishes with further latening of the anodal shock. Evidently elements contributing their potentials to the action

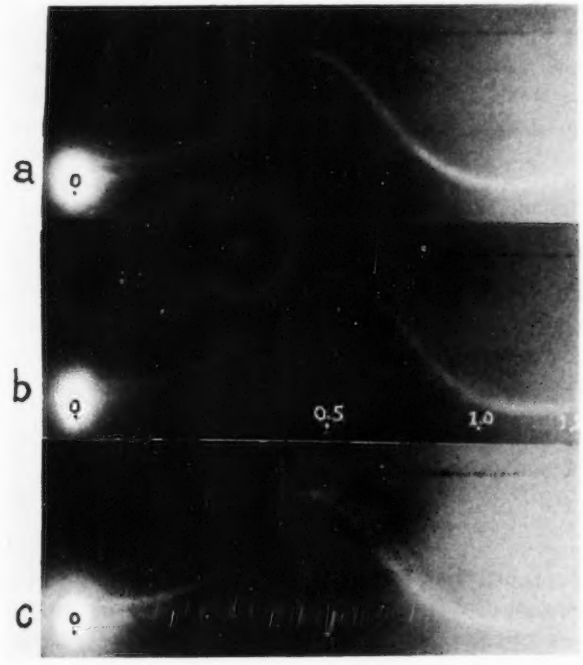

Fig. 8. (6/25/31) Contact prints of action potentials, conducted 24 mm., started by shocks equally submaximal, *a* from normal nerve, *b* from normal nerve cathodally polarized at the stimulating electrode with a 90 per cent rheobasic current. *c* shows the two action potentials printed over each other so as to bring out form and temporal differences. 28.2°C. Three panels, 1000ω. Time in sigmas.

potential as a whole can be made to drop out of the picture by an anodal shock placed as long as 0.4σ or more after a submaximal shock.

When the strength of the cathodal shock is such as to produce a maximal action potential, and not until then, the anodal shock loses its effect on the height of the action potential at all shock separations in excess, approximately, of the duration of the cathodal shock; and this is true no matter how strong the anodal shock be made. Indeed, the anodal shock then is

without effect not only on the height of the action potential, but also on its configuration. In these respects normal and polarized nerve act alike. The significance of this critical effect of shock strength will be considered later.

This evidence of a range of latencies that is definitely wider in polarized than in normal nerve is supported by action potential pictures. Conducted action potentials of the same submaximal height, one started in normal, the other in locally polarized nerve, differ very strikingly in appearance (see fig. 8). The latter, as compared with the former, has a longer and flatter rising phase, a shorter and more bulged falling phase; its center of area, therefore, and its crest are later. The end points of both are about synchronous. Obviously, the building stones of the polarized action potential are more massed toward its end. One gains the impression, which becomes practically a conviction in the case of action potentials that approach threshold amplitude, that the action potential starting in polarized

TABLE 1

Time, shock to beginning of conducted action potential in polarized as compared with normal nerve

EXPERIMENT	TEMPERATURE	POLARIZING CURRENT	SHOCK STRENGTH	TIME SHOCK TO ACTION POTENTIAL		
				Polarized	Normal	Difference
		per cent		σ	σ	σ
2/ 5/31	12.5	90	Thresh.	1.39	1.00	0.39
		90	Max.	0.92	0.85	0.07
2/14/31	22.0	90	Thresh.	0.90	0.74	0.16
3/ 7/31	11.8	90	Thresh.	1.42	1.18	0.24

nerve arrives at the lead later. It is difficult to decide this question definitely because the rising phase of the action potential initiated in polarized nerve has a much lower and more gradual gradient. A few of the measurements we have made using threshold stimuli are given in table 1. With the qualification mentioned, they show that the polarized action potential is the later of the two. In making this comparison it may be assumed that cathodal polarization has little if any effect on conduction rate (Bishop and Erlanger, 1926). Spread of the stimulating shock, though, might be a factor, for, since the action potentials compared are given the same amplitude (threshold, submaximal, or maximal), the shock delivered to the normal nerve always is the stronger of the two, and therefore stimulates further from the negative electrode. It may be asserted, however, (see Erlanger and Gasser, 1924) that the strength difference operative here could not account for "conduction time" differences of the magnitude of those observed in these experiments.

Bishop and Erlanger (1926), recording the action potential at or close

to the locus of the nerve whence it was started by the induction shock (maximal, in their experiments), found in polarized nerve an alteration in the configuration of the action potential similar in character to that exhibited in the present experiments by the conducted action potential, namely, a slight lengthening of the rising phase, a shortening of the falling phase, and, therefore, a shifting of the crest and center into later positions.

We have repeated the experiments of Bishop and Erlanger using, however, submaximal stimuli and leading directly from the point stimulated, which was also the cathode of the polarizing current. The strength of stimulation was adjusted so that the action potentials, normal and polarized, had the same height. Action potentials thus obtained are greatly deformed by the escape of the induction shock. Nevertheless, the records show that polarization definitely latens not only the crest of the action potential, as Bishop and Erlanger found, but also the end, the former, however, considerably more than the latter. Thus, in one of the experiments (at 14°C.), the difference in crest times was 0.23σ and in end times 0.13σ. With the same qualification that was made when considering the conducted action potential, it seemed that the unconducted action potential also began a trifle later in the polarized than in the normal nerve.

On the assumption that the unconducted action potential "must have had its constituent axon action waves almost exactly in the same phase", and without any additional information, it was natural for Bishop and Erlanger to have concluded, as they did, that cathodal polarization shortens the falling phase of the axon action potential but not the rising phase. Now, however, that it has been shown that the action potential conducted away from the locus of polarization has the same general configuration that it has at the locus of polarization, the alteration produced by the constant current can no longer be accounted for on this basis alone. For when the action potential is conducted away from the site of origin in the polarized region the axon potentials will resume their normal unpolarized configurations; and so also would the action potential as a whole. But any initial displacements of axon action potentials arising as a result of differences in latencies would be retained. In the light of present knowledge the modification of the unconducted (and also of the conducted) action potential by local cathodal polarization can be accounted for quite satisfactorily upon the assumption that it is due to a shortening by cathodal polarization not only of the falling phase but of the rising phase also, by invoking the evidence that the axon action potentials thus altered in configuration develop through a range of axon latencies that is wide enough to bring about the observed alteration in the figure formed by the summed axon potentials.

Discussion. The question now arises, is there any other way of accounting for the results we have attributed to differences in latencies?

The one thought that has occurred to us in this connection is that there may be a broad locus of stimulation, impulses consequently starting in different fibers at positions sufficiently widely separated spacially to account for the time spread observed. There are difficulties, however, in the way of this concept. Even if the spread included the entire inter-electrode distance the time range, determined by calculation based on conduction rate, still would be insufficient to account for the phenomena we attribute to differences in the recording times of the axon action potentials. A more potent objection to this view is the fact that the efficacy of an anodal shock in reducing the response to a submaximal cathodal shock decreases as the interval between the shocks increases. If latency to the cathodal shock were not the responsible factor, the anodal shock should be without effect when applied outside the utilization period of the former, that is to say, later than about 0.06σ; but an anodal shock has an effect under appropriate conditions even after ten times this interval.

Experiment has shown that the preterminal period, the time elapsing between the testing shock and the constant current break, and the maximum separation of cathodal and anodal shocks effective in reducing the action potential height are the same, and are about equal to the time to maximum of the action potential recorded under the same conditions. This result, it is easy to show, is a necessary consequence of the latency hypothesis, provided the latency range be greater than the time to maximum of the action potential, and experiment indicates that it is. The action of both the anodal shock and the constant current break in this respect can best be regarded as a prompt lowering of the *responsivity* of the fibers to the level where the local process that has been started by the cathodal shock, but has not yet produced a response, fails to mature. Since the latency range is wider than the ascending phase of the action potential the amplitude of the action potential will be cut down when the anodal shock or constant current break falls between the activating shock and the crest of the action potential and thus eliminates some of the axon action potentials that contribute to the crest of the composite potential.

The one observation that it is difficult to fit into the view that the constituent fibers of a nerve respond to an induction shock, not all at once, but with latencies ranging from the end of the utilization period of the shock to some point later than the crest of the action potential, is the suddenness of the development of the inefficacy of an anodal shock, placed beyond the end of the utilization period of the cathodal shock, at the point where the cathodal shock becomes strong enough to yield a maximum action potential. The only way out of this difficulty that is consistent with the hypothesis of latencies is to assume, as we did above, that the anodal shock acts not by instantly annulling reactivity but, like the ending of the constant current, by lowering local responsivity evenly for a considerable

period, not merely for the duration of the shock, to a *level* where some of the local processes started by the cathodal shock fail to mature. When the cathodal shock is well above maximum neither the break of the constant current nor the anodal shock can lower responsivity by enough to interfere with the local firing off process. Under these circumstances the anodal shock can affect the response produced by the cathodal shock only

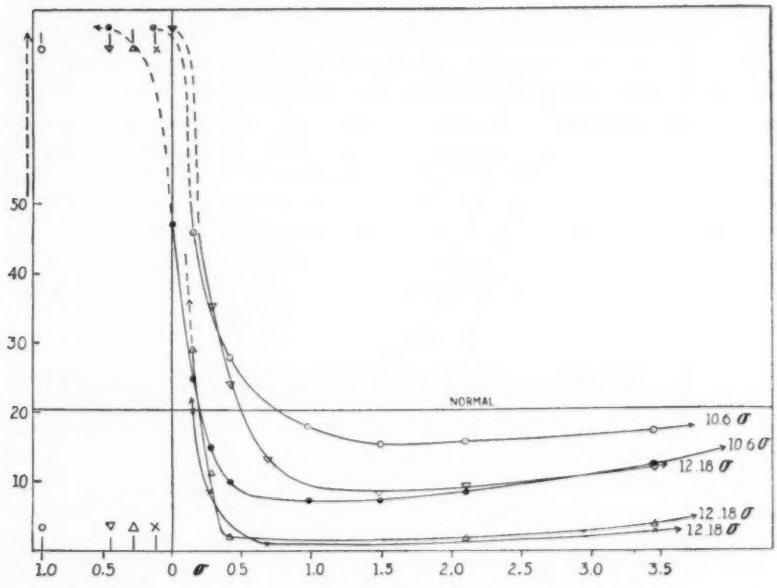

Fig. 9. (2/12/31) Irritability changes (at the cathode) associated with the break of a constant current of various durations. Current strengths all 50 per cent of threshold. 13.0° – 13.8°C. Current durations: ● = "long," ○ = 0.94σ, ▽ = 0.44σ, △ = 0.28σ, × = 0.12σ. Ordinates, amplitude of action potential; abscissae, sigmas, zero being the constant current end. The point at which the action potential begins to diminish in height on moving the testing shock toward the end of the constant current is a separate determination in each case. An uncorrected shift in the base line during the experiment makes curves △ and × 1 to 2 mm. too low.

when it directly counteracts the current flow determined by the latter, that is to say, only when it acts during the utilization period of the cathodal shock.

It is concluded on the basis of this discussion that the diminution in the height of the action potential that occurs as the cathodal testing shock approaches the end of a "long" period of subthreshold cathodal polarization is due to differences in latencies of the responding fibers wider than

the time to maximum of the action potential as it develops under the stimulating electrode.

The summation interval. It has been seen that upon carrying the submaximal testing shock later and later in a subthreshold constant current the resulting action potential begins to diminish in height before the polarizing current terminates. This diminution in height, beginning in the preterminal period, gathers speed gradually and attains its maximum velocity

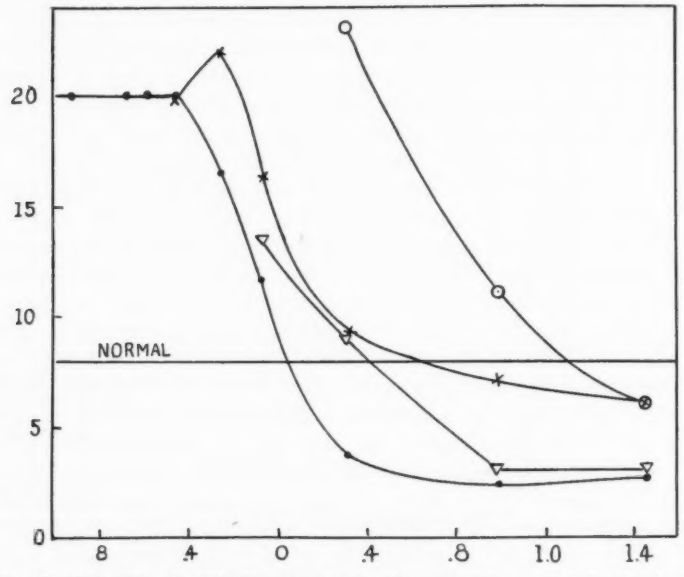

Fig. 10. (1/31/31) Irritability changes (at the cathode) associated with the break of a constant current of various durations. 11.7° – 12.8°C.
● = "long" current, 90 per cent of rheobase.
× = 0.58σ current, 90 per cent of rheobase.
▽ = 0.20σ current, 90 per cent of rheobase.
○ = 0.20σ current, slightly below threshold.
Ordinates, amplitude of action potential; abscissae, time in sigmas, zero being constant current end.

at about the time the testing shock and the opening of the constant current coincide (figs. 9 and 10). At this time the irritability still is above that of the nerve when unpolarized; but it continues to fall and with undiminished speed until it passes below the normal level well into the period of postcathodal depression. In other words, there is, following the break of a "long" constant current, a *summation interval* if the latter be defined as the period lying between the constant current break and the point where

the descending irritability crosses the level of normal irritability. It is, however, exceedingly brief. In cold nerve (12°-13°C.) it probably is not over 0.2σ, and in warm nerve it is so brief that it scarcely can be measured when the constant current has about 90 per cent of the rheobasic voltage. We have not made any observations on the summation interval following the break of a long constant current of higher than rheobasic voltage after complete recovery from the initial response.

We have, however, measured the summation intervals following constant currents of different, subthreshold strengths, and various durations. The conditions become rather complex in these experiments when the durations fall within the range of the irritability changes incident to the constant current make. For an exact analysis of the relation of the summation intervals to these initial changes it would be necessary to have for each current strength an observation at each of the significant durations, and the latter could be ascertained only by reference to a curve showing the initial changes in irritability produced by a long constant current in the same preparation. Such a systematic study we have not made. The observations we have, however, suffice to indicate the general trend of these interrelations.

When the current strength is kept constant at say 90 per cent of the rheobase while the current duration is changed it is found that the longest summation interval corresponds with current durations which presumably would place the constant current break in the plateau stage of the initial irritability complex; briefer currents again yield shorter summation intervals, as short, when the currents are very brief, as those following long constant currents. The same result, qualitatively, is obtained when, for each of the current durations, the current strength is adjusted so as to have the same value, relative to threshold *provided the value be not too high.* Figure 9 depicts the results of such an experiment in which the current strength was maintained at 50 per cent of the threshold value. The temperature remained close to 13.4°C. throughout. The summation intervals following currents lasting, in the five successive determinations, somewhere between 20 and 40 σ, 0.94, 0.44, 0.28 and 0.12 σ were 0.20, 0.78, 0.50, 0.20 and 0.16 σ, respectively; the summation interval is maximum when the current is broken at about what would be the plateau stage of the initial irritability changes produced by a long constant current.

When the constant current strength is held at a high and constant level relative to the threshold at each duration, it is found that as the durations are reduced the summation intervals do not diminish again when the current becomes brief; instead, they may actually continue to increase. This result may be illustrated by the data of another experiment (fig. 10, temperature 12°C.). In this case a long constant current at 90 per cent of the rheobase had an estimated summation interval of 0.1 σ. Upon

shortening the constant current to 0.58 σ and to 0.20 σ, but not altering its strength, the summation intervals became 0.6 and 0.4 σ, respectively. Now, increasing the strength of the 0.20 σ current to 90 per cent of its threshold value increased the summation interval to 1.2 σ.

Though these and other similar observations, as has been said, have not been carried out systematically they nevertheless can be regarded as showing very definitely that the duration of the summation interval following the break of a constant current depends upon both the phase of the initial irritability transient in which the break falls and the strength of the polarizing current. When the constant current is kept at a constant, subrheobasic level, interruption during the steady state leaves a summation interval so brief that it can be recognized with certainty only in cold nerve; the longest summation intervals are those following interruption during the plateau stage; earlier interruption again reduces the summation intervals until, with very brief constant currents, they may be as brief as those following interruption during the steady state. But if such a very brief constant current is now increased from rheobasic strength to the point where it is just subthreshold the summation interval becomes long, longer, perhaps, than the longest that can be obtained with just subrheobasic currents of any duration.

Discussion. These observations indicate that the duration of the summation interval is not determined primarily by the irritability obtaining at the moment of the break, as measured by an induction shock; for the irritability during the maintained stage relative to normal is about a quarter less than that obtaining during the plateau stage, but the summation interval determined by breaking the constant current during the plateau stage is at least six times as long as that following the break during the maintained stage. Presumably, therefore, the summation interval is an expression of a dynamic rather than of a static process; the height of the maintained polarization plays a very minor rôle. The observations prove, furthermore, that the dynamic process concerned is related primarily to the changes that are initiated by the rising potential; the break of the constant current during the steady state contributes little if anything to the summation interval. It is even conceivable that the sudden break tends to abolish a summation interval that is potential at the moment.

It would be interesting to compare the maximum summation interval following the break of a constant current with the summation interval determined by a brief induction shock. In the absence of a systematic investigation of the summation intervals following the break of brief constant currents we cannot be sure that we have succeeded in determining that maximum. With the information now available, however, it is obvious that the two maxima are of the same order of magnitude; thus at

12°C. the maximum constant current summation interval we have encountered has been 1.2 σ; it was determined by a 0.2 σ constant current. And at 12.8°C. we have recorded a summation interval of 1.6 σ following a break shock lasting 0.04 σ. In both instances the sensitizing currents had a strength of 90 per cent of threshold.

The results we have obtained with constant currents of brief duration confirm those recorded in the preceding paper in showing that the length of the summation interval within limits is independent of the duration of induction shocks, but is related to shock strength. The fact that the

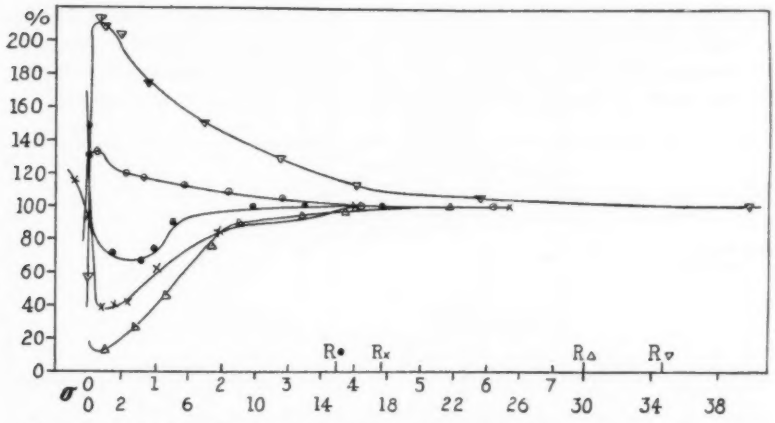

Fig. 11. (7/24/30) The irritability responses associated with the break of a long constant current. Ordinates, amplitude of action potential in per cent of normal. Abscissae, sigmas, upper scale for dot, cross and circle curves; lower scale for △ and ▽ curves; zero marks the constant current break.

Dots and crosses; at the cathode, 30.0°C.

Circles at the anode, 30.0°C.

△; at the cathode, 15.0°C.

▽; at the anode, 13.0°C.

The *R*s mark the ends of the corresponding relatively refractory phases.

break of the constant current contributes little if anything to the summation interval accounts, at least in part, for this result.

The negative phase. The final stage following the break of the constant current is the well-known phase of postcathodal depression. In cold nerve (12° to 15°C.) the irritability, plotted in the form of a curve, makes a broad sweep downward reaching minimum somewhere between 0.5 and 3.0 σ after the break, usually between 1 and 2 σ. At room temperature the low point is found in the vicinity of 0.3 to 0.4 σ after the break. The return to the normal level is much slower, and, as in the case of induction

shocks, occupies about the same time at any given temperature above 12° to 15°C. as the recovery from relative refractoriness (see fig. 11).

In order to convince ourselves that this depression in irritability actually is the result of prior cathodal polarization, in a few experiments data have been obtained for the plotting of the irritability changes developing at the anode when a subrheobasic constant current is broken. Excepting possibly some quantitative differences, which we are not in a position to insist upon at the present time, the irritability curves thus obtained are the postcathodal curves inverted (see fig. 11). If confirmation were needed of the conclusion recorded in the preceding paper to the effect that the depression of irritability following the summation interval produced by a subthreshold cathodal induction shock is a postcathodal depression, it is supplied by these observations on the irritability changes developing at the anode and cathode following the break of a constant current.

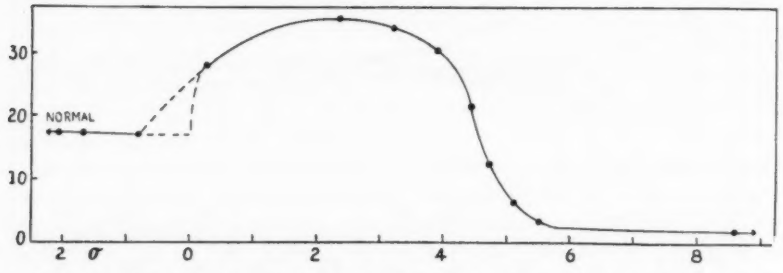

Fig. 12. (7/26/30) The irritability changes (at the cathode) accompanying polarization by a current rising at less than the liminal rate. Ordinates, amplitude of action potential in millimeters; abscissae, time in sigmas. The current begins to rise at zero.

The depression following the break of a subrheobasic cathodal polarization in many ways seems to resemble the fall to the maintained level following the plateau stage developed by the make. It is extremely, difficult, however, to obtain exactly comparable data, and we have not made a special effort to get them. The resemblances, stated in general terms, are these: both vary alike with the polarization strength, and in respect to depth and duration they are of the same order of magnitude under the same conditions. Therefore, if the drop to the maintained level is the expression of the development of depression due to the action of the current on the nerve, the postcathodal depression, as has been mentioned before, could very well be accounted for on the basis of exposure of the prior depression by the disappearance of cathodal enhancement of irritability.

The liminal gradient effect. Finally, two consistent observations have

been made on the changes in irritability occurring in nerve at the cathode of a current that is made to increase to a relatively high intensity but at such a rate that stimulation fails to occur. The results of one of the experiments are shown in figure 12. The wiring diagram (fig. 13) shows that with the break of the short circuit the potential drop through the nerve increases as the 1 microfarad condenser charges through something less than 50,000ω. This condenser would acquire half its charge in something less than 37σ. Since the significant part of the experiment is completed

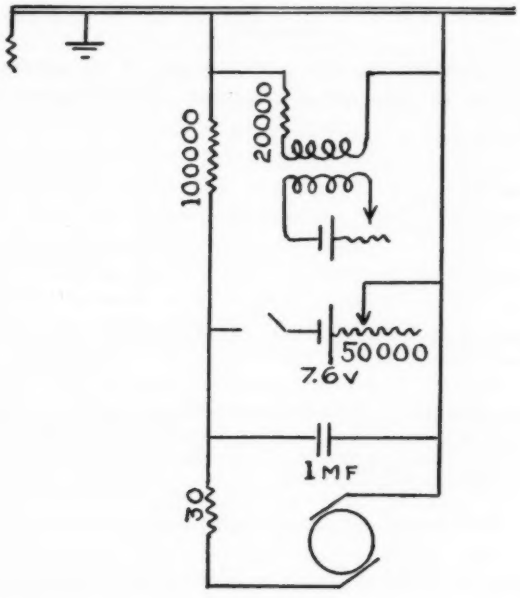

Fig. 13. (7/26/30) Diagram of circuit to test the irritability changes determined by a current rising at less than the liminal rate.

in about 6 σ, it is obvious that, practically speaking, the applied potential to which the nerve reacts grows linearly. A battery of 7.6 volts was used as the source of current. As the strong currents employed rapidly damage the nerve it was necessary to carry through the experiment just as quickly as possible. Therefore, the measure of irritability used was the height of the action potential.

The curve shows that the irritability of the nerve first rises, but at a decreasing rate, and then falls, more rapidly than it rose, to a level far below normal. From this state of subnormal irritability the nerve recovers

slowly and incompletely; obviously it is in a state of cathodal depression. The experiment convincingly shows what has been inferred by others (for example, Bishop, 1928 a), that one of the reasons for the failure of nerve to respond to a current rising in intensity at less than a certain rate is the ultimate supervention of cathodal depression. It is of interest to point out here that the irritability curve recorded in this experiment has the same general form as the one (see fig. 5) expressing the relation between the irritability of nerve obtaining during the steady state following the make of a constant current and the strength of the constant current.

SUMMARY

By methods involving the direct registration of the amplified action potential with the cathode ray oscillograph, determinations have been made of the "irritability" of nerve as affected by short periods of polarization.

The alteration in irritability at the cathode of a constant current of just subrheobasic strength, acting on nerve at a temperature of about 15°C. (for example), is shown diagrammatically in figure 14. (The Q_{10} of many of the features is about 1.6.)

With the make the observed irritability (heavy curve) rises quickly to a plateau which, at about 2 σ, is terminated abruptly by a decline in irritability, concave upwards, which approaches asymptotically the level maintained by polarization.

This picture can be closely matched by the algebraic addition of two curves, one (I) rising quickly, the other (D) falling slowly, from the level of normal irritability; and evidence is given indicating that the falling curve (D) represents an action of the current depressing irritability, the rising curve (I) an action of the current enhancing irritability, both initiated simultaneously.

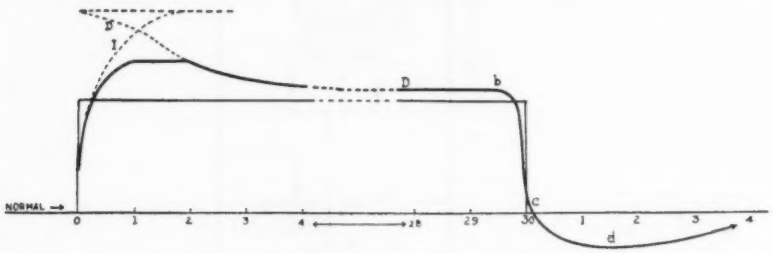

Fig. 14. Diagram summarizing the irritability responses at the cathode of a long (30σ) subrheobasic current. The rectangular figure gives the position of the make and the break of the polarizing current. Ordinates, irritability; abscissae, time in sigmas. For further description see text.

The change in the configuration of the action potential when nerve is cathodally polarized is consistent with the conclusion that the rising as well as the falling phase of the axon action potential is abbreviated by polarization.

The decline of the irritability curve, beginning (at *b*) before the constant current breaks, seems to be determined by axon latencies ranging in duration, with shocks of all strengths, between the utilization period of the shock, or slightly thereafter, and a value greater than the time to maximum of the action potential. This range is wider in polarized than in normal nerve.

The existence of a summation interval following a shock, signifying, as it does, that the nerve is held in a state of enhanced irritability for an appreciable period in the absence of the current, suggests the possibility that we are dealing here with a device which puts the nerve in a position to respond to a brief current or other stimulus at any time within the range of the summation interval, which then would be the range of latencies also.

The break of a long, slightly subrheobasic, constant current is followed by an almost negligible summation interval, *c*. As this same current is shortened in duration (for the sake of clearness, from behind) the summation interval increases to a maximum that is reached when the break occurs during the plateau, and diminishes again with briefer currents. But if, as the current is shortened, its strength be maintained at a just subthreshold level, the summation interval does not shorten, it may even lengthen, as the current duration becomes less than the time to the plateau.

It is concluded that the summation interval is determined by a dynamic rather than by a static process, the break of the current probably contributing little if anything to it.

The break of the constant current is followed by the period of postcathodal depression, *d*, which, very generally speaking, varies in depth and duration as the depression following the plateau.

The postcathodal depression has about the same duration as the relatively refractory phase.

In nerve that is being polarized cathodally at less than the liminal rate, irritability first rises, but finally falls and becomes subnormal due to cathodal depression.

BIBLIOGRAPHY

BISHOP. 1928a. This Journal, lxxxv, 417.
1928b. This Journal, lxxxiv, 417.
BISHOP AND ERLANGER. 1926. This Journal, lxxviii, 630.
ERLANGER AND GASSER. 1924. This Journal, lxx, 624.
SCHAFER. 1900. Textbook of physiology, ii, 498.
WERIGO. 1883. Pflüger's Arch., xxxi, 417.

THE AFFERENT PATHS OF NERVES INVOLVED IN THE VOMITING REFLEX INDUCED BY DISTENTION OF AN ISOLATED PYLORIC POUCH

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That the sensory fibers involved in the vomiting reflex are follow pathways of the autonomic nervous system is not accepted by all investigators. In the progress of experimental work that involved injecting solutions into isolated pyloric pouches of dogs, an opportunity presented itself to study this problem.

It was found that distention of pyloric pouches until the pressure within the pouch reached a definite level invariably induced vomiting. The pouches were prepared by a modified two-stage Polya resection type of operation, which involved complete section of the wall of the stomach through the so-called prepyloric and the pyloric sphincters. Ivy and Oyama (1921), and Lim and Dott (1922) consider that such pouches are denervated of vagus fibers. As the vomiting was invariable, it appeared that either the afferent fibers passed over the pathways of the sympathetic nerves, or that the supposedly denervated pouches contained vagus fibers.

In the past, the autonomic nervous system was believed to be entirely made up of efferent fibers. Gaskell's view that the sympathetic system is made up of two elements, one of which is motor cells that have migrated from the central nervous system, and the other, their communicating fibers, is well known. The sympathetic system does not contain afferent fibers, and all reflex arcs involving this network have fibers of the central nervous system on the afferent side.

Langley (1903) stated his belief that all nonmedullated fibers are efferent and that the sympathetic system is not complete but consists purely of excitor neurones. Langley and Anderson concluded that the two supposed examples of sympathetic reflex arcs, namely, the salivary reflex through the submaxillary ganglion and the vesical reflex through the inferior mesenteric ganglion, are so-called axon reflexes and not true reflex arcs.

Miller (1911) studying the pathways involved in the emetic action of mustard, reached the conclusion that the vagus nerve is the only afferent pathway, as vagotomy definitely inhibited that action. Tilney and Riley

expressed the belief that the vagus and glossopharyngeal nerves are the afferent pathways of vomiting reflexes.

Ranson (1918) stated that satisfactory evidence is lacking of afferent neurones having their cell bodies in the sympathetic system, and that all visceral reflex arcs must pass through the central nervous system, except local myenteric reflexes within the wall of the intestine.

Lewandowski (1919) was probably the first investigator to imply that the autonomic system included afferent fibers from the viscera. These, if they existed, might be the afferent fibers in vomiting reflexes.

Hatcher and Weiss (1922), following the work of Hatcher and Eggleston (1912) who proved that the emetic action of the digitalis bodies is central, believed that reflex nausea and vomiting caused by these bodies is through direct action on the heart, stimulating afferent fibers in that organ which pass along the pathways of the sympathetic nerves, but also pass in part by the vagus nerve to the medullary vomiting center. This belief was based on the results of the actions of drugs in acute experiments, and as such are not fully accepted by all investigators. Alvarez (1928) accepted fibers passing along the autonomic system as being afferent fibers in the reflex arc.

METHODS AND RESULTS. When it had been definitely established that distention of four of these pyloric pouches invariably produced vomiting, the pressure within the pouch necessary to initiate the reflex was measured by a *T* apparatus. One arm of the crosspiece was connected to a retention catheter in the pouch, the other to a mercury manometer, and the remaining portion of the *T* was used as the water supply to the system. It was found that a pressure of from 30 to 35 mm. of mercury was necessary to initiate the reflex. This remained remarkably constant, regardless of the solution used. Water, dilute acids, alkalis and mustard were used.

Vagotomy was done on three of the four dogs by the transpleural approach through an incision made in either the seventh or eighth intercostal space as suggested by Beaver and Mann. These and other operative procedures were carried out under ether anesthesia, and sterile technic. A section of the right vagus nerve several centimeters long was resected to preclude regeneration, the mediastinum was broken posteriorly, and the left vagus nerve was similarly resected.

Two of the three dogs so prepared could no longer be induced to vomit by distention of the pouch, although pressure within the pouch was raised to about twice that which invariably induced emesis before vagotomy. Distention of the pouch of the third dog continued to induce vomiting. The right sympathetic chain was evulsed from this dog, using a transpleural approach, from a point just below the diaphragm to well up into the thorax, six rami being removed. This procedure had no effect on the vomiting reflex. Similarly, removal of the same length of the left sympa-

thetic chain did not have any effect on the reflex. It was suggested that there might be some fibers in the right phrenic nerve which were acting as afferents to the arc, and right phrenicectomy was done in the neck, but the status of the reflex did not change.

At this stage it was believed that possibly a branch of the vagus nerve had been left intact during vagotomy, and the vagi were explored through the old right intercostal incision. It was found that the left vagus had split into two branches at a point higher than usual, and that only one branch had been resected. The other branch was resected, following which vomiting could no longer be induced by distention of the pouch. A splendid control had been inadvertently established through a fortunate error in technic. In all the experiments, therefore, vagotomy prevented the invariable reflex of vomiting, as produced by distention of a pyloric pouch.

Pouches of the fundus of the stomach were distended many times in unsuccessful attempts to induce emesis, as were pouches of the pylorus and fundus that had been transplanted into the subcutaneous tissue after the method of Ivy and Farrell (1925). It is not to be expected that vomiting could be induced by distending these pouches, as they are truly denervated, and there is no evidence at present that vomiting can be induced through a humeral mechanism.

COMMENT. In the light of the views of various observers it is not surprising to find that the afferent arc in this particular vomiting reflex is purely through the vagus. The work of Hatcher and Weiss (1922) is the basis of the idea that the fibers of the autonomic system may act as afferents in this arc. In their experiments the heart is the site of the stimulus, and it is possible that afferent impulses may leave this organ along tracts of the sympathetic nerve fibers. Evidence is not available to support the idea that a stimulus arising within the gastro-intestinal tract can induce vomiting by producing sensations that pass along autonomic nerve fibers.

It is interesting to note that the pouches which were supposedly free of intact fibers of the vagus actually were not completely denervated. Undoubtedly a good proportion of these fibers have been severed, but enough remained for the purpose of setting up at least this reflex arc when stimulated. What effect this may have on experimental work with secretion or pyloric pouches so prepared is problematic.

Why distention of the antrum of the stomach will induce vomiting, whereas distention of the fundus will not, is as yet unsolved. The fibers of the vagus probably enter the pouches by either or both of two paths with the sympathetic nerves along blood vessels, or with a branch of the vagus from the liver to the pylorus. Bayliss and Starling (1899) have shown that the vagus gives a branch to the solar plexus, and it is possible that some of these fibers are distributed to the wall of the stomach along with the nonmedullated fibers. Whether these fibers do not reach the

fundus, or whether the branch of the vagus from the liver carries all the impulses from the antrum which induce emesis has not been determined. The significance of distention of the antrum as a mechanism of inducing vomiting is also unknown. It would seem that it would be one of the most physiologic stimuli acting on the intact stomach, but there is no evidence that it is a more important stimulus to vomiting than any other stimulus that arises in other organs, or that is induced either directly or indirectly by the actions of drugs and other substances on the vomiting center.

SUMMARY

Reflex vomiting can be induced by distending an isolated pyloric pouch until the pressure within the pouch rises above a certain constant level which can be definitely measured.

The afferent side of this reflex arc is entirely through the vagus nerves.

Supposedly denervated pouches contain enough intact vagus fibers to carry these afferent impulses.

BIBLIOGRAPHY

ALVAREZ, W. C. 1928. The mechanics of the digestive tract. New York, Paul B. Hoeber, Inc., 447 pp.

BAYLISS, W. M. AND E. H. STARLING. 1899. Journ. Physiol., xxiv, 99.

BEAVER, M. G. AND F. C. MANN. 1931. Technic for opening and closing the chest in experimental surgery (in press).

GASKELL, W. H. 1920. The involuntary nervous system. New ed., London, Longmans Green and Co., 178 pp.

GOLDBERG, S. L. 1931. Intrinsic regulation of gastric acidity (in press).

HATCHER, R. A. AND C. EGGLESTON. 1912. Journ. Pharm. Exper. Therap., iv, 113.

HATCHER, R. A. AND S. WEISS. 1922. Arch. Int. Med., xxix, 690.

IVY, A. C. AND J. I. FARRELL. 1925. This Journal, lxxiv, 639.

IVY, A. C. AND Y. OYAMA. 1921. This Journal, lvii, 51.

LANGLEY, J. N. 1903. Brain, xxvi, 1.

LANGLEY, J. N. AND H. K. ANDERSON. 1894. Journ. Physiol., xvi, 410.

LEWANDOWSKI, M. 1924. Quoted by R. A. HATCHER. Physiol. Rev., iv, 479.

LIM, R. K. S. AND N. M. DOTT. 1922. Quart. Journ. Exper. Physiol., xiii, 159.

MILLER, F. R. 1924. QUOTED by R. A. HATCHER. Physiol. Rev., iv, 479.

RANSON, S. W. 1918. Journ. Comp. Neurol. xxix, 305.

TILNEY, F. AND H. A. RILEY. 1921. The form and functions of the central nervous system. New York, Paul B. Hoeber, Inc., 1020 pp.

THE SECONDARY RISE IN BLOOD PRESSURE FOLLOWING PERIPHERAL SPLANCHNIC NERVE STIMULATION

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I. PREVIOUS WORK. Dreyer (1898) was the first observer to demonstrate that electrical stimulation of the peripheral end of the splanchnic nerve increases the output of adrenalin from the adrenal gland. In 1908 Lehndorff took blood pressure tracings while stimulating the peripheral splanchnics and obtained a curve consisting of a first and then a second rise. However, he did not mention adrenalin in the interpretation of his curves. Later Anrep (1912) stimulated these same nerves and observed an increase in the rate and tone of the denervated heart coincident with this secondary rise in blood pressure. When he clipped the adrenal veins there was no secondary rise and no cardiac change. He concluded then that "the secondary rise and all concomitant phenomena were due to the discharge of adrenalin into the circulation, and were absent after extirpation of the adrenal glands."

On the other hand Gley and his co-workers (1913, 1916, 1917, 1921, 1923) reported than in most dogs the secondary rise occurred even after double adrenalectomy. The same was found to be true of the cat, so that on the whole they were not in accord with the idea that the secondary rise was due to secreted adrenalin.

Pearlman and Vincent (1919) found on both cats and dogs that the blood pressure curve of splanchnic stimulation was seriously modified by elimination of the glands, although the total rise in pressure after adrenalectomy might even be greater than before. Their typical control curve consisted of a sharp primary rise with a step about half way up, then a "dip" followed by a secondary rise. After removing the glands they found the dip to be absent, so attributed its presence before adrenalectomy "to the discharge of adrenin in such doses as to produce a depressor effect."

Thus, while all workers are agreed that the primary rise is due to constriction of the splanchnic blood vessels from nervous activation of the vessel musculature, these contradictory interpretations of the secondary rise exist: 1, it is due entirely to pressor effects of adrenalin secreted by the adrenals; 2, it has no relation to the adrenal glands; 3, the dip between rises is due to the secretion of "depressor doses" of adrenalin.

II. PRESENT EXPERIMENTS. Because of this confusion about the cause of the secondary rise the present work was undertaken. Observations were made on sixty dogs under ether or barbital anesthesia. Through a midline incision the right splanchnic nerve was isolated and cut and the right adrenal gland removed. The left splanchnic was then isolated and cut and the peripheral end stimulated with either plain or shielded electrodes. Figure 1A is a tracing showing the effect on carotid blood pressure, of such a stimulation, the left adrenal still being intact. This kind of secondary rise coming just as the primary vaso-constriction has ceased we believe to be due mainly to the secretion of adrenalin by the adrenals because; 1, it is obtained in this form only when at least one adrenal is intact; 2, it is always modified (but not obliterated) by adrenalectomy or by occluding the lumbo-adrenal veins; 3, it is only with such a rise that dilatation of the sensitized pupil was seen.

After the second adrenal has been removed without injury to the splanchnics curves as in figures 1B and 1C were usually obtained. The curve is seen to consist of a sharp primary rise, a dip, and then a secondary rise with a subsequent drop to the pre-stimulation level. Frequently the secondary rise following the dip was higher than the primary rise.

Often even before the second adrenal was removed curves similar to those of figures 1B and 1C were obtained. This may probably be explained on the basis of the work of Elliott (1912), who showed that ether, chloroform, and other anesthetics exhausted the adrenalin supply of the glands if the splanchnics were intact. If then the adrenals are exhausted of adrenalin even before the control stimulation, the blood pressure curve before adrenalectomy would have the same appearance as after removal of the glands. Thus when Gley believed that adrenalectomy did not modify the blood pressure curve of splanchnic stimulation it may have been because he did not obtain the true adrenalin secondary rise before extirpation, simply because the anesthesia had depleted the adrenalin store of the gland. Furthermore Lehndorff's curves (1908) in his figures 2 and 6, both obtained before adrenalectomy, are almost identical with our curves in figures 1A and 1C respectively, the former taken before adrenalectomy, the latter, after. His curve of figure 6 is probably entirely independent of the adrenal glands although these were anatomically still intact.

Stimulation of either splanchnic after bilateral adrenal extirpation usually elicits similar curves, but occasionally one nerve fails to give a typical result. Rarely neither splanchnic gives the secondary rise, unless there has been undue trauma, or injury to the nerve, in which case the primary rise is also poor or even entirely lacking. The kind of anesthesia used is also a factor, for secondary rises were obtained more often when barbital was used.

III. POSSIBLE MECHANISMS INVOLVED. Concerning the cause of the

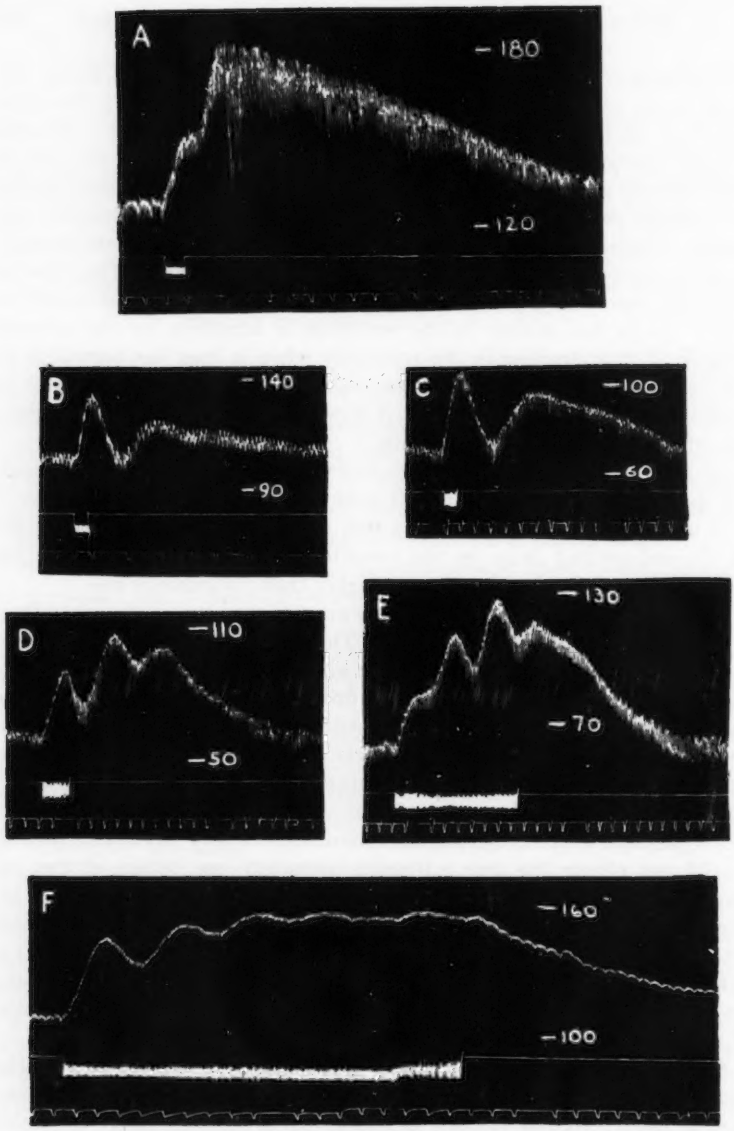

Fig. 1. Carotid blood pressure curves from peripheral splanchnic nerve faradization. Figures indicate millimeters of mercury. Signal marks application of stimulus. Time intervals are five seconds. A. Control. Right adrenal has been removed, left adrenal still intact. Stimulation of left splanchnic. B, C. Residual secondary rises after bilateral adrenalectomy. D, E, F. The same, except that several rises are seen.

residual secondary rise after bilateral adrenalectomy these mechanisms were considered. 1. *Reflexes.* Perhaps the elevated blood pressure of the primary rise might stimulate afferent nerves which would reflexly affect the heart or vaso-motor system in such a way as to bring about a second elevation. In adrenalectomized animals with double splanchnectomy, double vagotomy, or spinal cord section (at cervical 7) the secondary rise still persisted. Furthermore, this effect was observed immediately after cord section in the period of spinal shock, when presumably all reflexes are extremely poor or entirely abolished.

2. *Direct cardiac stimulation.* In addition to reflexes acting on the heart, changes in output might occur either through substances elaborated elsewhere than in the adrenals, or through changes in coronary pressure (Guthrie and Pike, 1907). The former possibility seemed to be excluded on the basis of experiments by Cannon (1922). The slight acceleration of the denervated heart which occurred on splanchnic stimulation after double adrenalectomy was practically abolished after section of the nerves

TABLE 1

Average heart rates accompanying changes in blood pressure following peripheral splanchnic stimulation in 12 adrenalectomized dogs

	HEART RATE DURING:					
	Control	Primary rise	Primary fall	Secondary rise	Secondary fall	Control
Average of 22 cases of stimulation...	127	134	128	137	119	121
Single stimulation in dog 32.....	86	55	154	84	87	90

to the liver. In our experiments the secondary rise in blood pressure after adrenalectomy was not abolished by so denervating the liver. In later work Cannon (1931) reports instances of cardiac acceleration from splanchnic stimulation even after adrenal extirpation and liver denervation.

However, the possibility of cardiac acceleration being a factor responsible for the secondary rise can be eliminated from the following considerations. The average heart rates during the different stages of the blood pressure curve in twenty-two instances of stimulation are shown in table 1. It must be borne in mind that these rates do not refer to denervated hearts and are thus not comparable to Cannon's data on the rate of the denervated heart. The question involved here is whether the intact heart increases its rate sufficiently to account for the secondary blood pressure rise.

In general it will be seen that the heart rate changes are in the same direction as the blood pressure variations. In six individual cases, however, there was an increase in rate with the primary fall, and a decrease

with the secondary rise. The heart rates in the most striking of these cases appear in line 2 of table 1. Furthermore, changes of rate of the order of magnitude indicated in line 1 of this table would probably be inadequate to account for the great changes in pressure sometimes observed. It is more likely that the increases in rate usually seen during the secondary rise are the result of the increased blood pressure and consequently improved coronary circulation, rather than the cause. For increases in rate equal to those of the secondary rise are also seen during the primary rise, which is admittedly a purely vaso-constrictor phenomenon.

The possibility must also be considered, of an increased stroke volume resulting from some change in the heart (e.g., increased coronary pressure) induced by the primary blood pressure elevation. Such an action would seem to be excluded on the basis of the fact that, although stimulation of either splanchnic elicited excellent and equal primary rises, presumably leading to equal changes in coronary pressure, yet only one splanchnic gave a secondary rise, in some animals.

3. *Peripheral mechanisms.* That this blood pressure curve was not due to any sort of rhythmic after-discharge either from the nerve at the point of stimulation, or from the coeliac ganglion, was shown in the following way: After cessation of stimulation, when the primary rise was at its height, the splanchnic nerve was cut about a half inch peripheral to the site of stimulation in such a way that the coeliac ganglion was also removed. This procedure had no effect on the curve.

We may conclude from all the above experiments that the secondary rise after adrenalectomy is due to the activity of some peripheral mechanism, some splanchnic nerve-end organ complex. The following of these were considered:

a. Hepatic pressor substance. It seemed reasonable to expect that the cardio-accelerator substance which Cannon (1922) demonstrated to be liberated from the liver on splanchnic stimulation is also a pressor substance. Elsewhere is mentioned the fact that denervation of the liver as described by Cannon resulted in no change in the blood pressure curve in the adrenalectomized animals.

b. Splenic contraction. With the idea of removing any blood pressure changes which might be induced by the contraction of the splenic smooth muscle, the spleen was temporarily cut out of the circulation after adrenalectomy. The splanchnics were then stimulated, and the typical curve with the double rise was again seen.

c. "Adrenalin" from chromaffine tissue. Could this secondary rise be due to the liberation of an adrenalin-like substance from the chromaffine bodies known to exist in the splanchnic region? If this were true, the presence of the material in the blood stream should produce effects on other

tissues than the blood vessel musculature. In 1904 Meltzer removed the superior cervical ganglion in rabbits and cats and after a few days found the pupil to be sensitive to adrenalin in amounts too small to affect the pupil of the normal eye. Elliott (1912) found that in doubly adrenalectomized cats with one superior cervical ganglion removed, stimulation of the splanchnics led to slight pupillary dilatation in a few cases. He suggested that this might result from adrenalin liberated by the paraganglia or by the actual processes of nervous excitation, or from other metabolites. Hartman (1923), using the supersensitive eyes of cats, reports little or no response on stimulation of the splanchnics after adrenalectomy. Stewart and Rogoff, however (1916), in the same experiment on dogs, found that the response of the partially denervated eye was due solely to adrenal adrenalin, and was absent when the venous path from the glands was blocked. Our results on dogs confirm those of Stewart and Rogoff. The discrepancy between the positive and the negative results of these investigators, may possibly be explained on the basis of species differences. Perhaps the pupil of the cat's eye is more sensitive to adrenalin-like substances than that of the dog. Or perhaps splanchnic stimulation leads to the liberation of more such material in the cat than in the dog. At any rate, the negative experiments do not preclude the possibility of a pressor, adrenalin-like substance being responsible for the secondary rises.

Nor does the fact that the secondary rises in figures 1B and 1C (after adrenalectomy) appear later than in figure 1A (control) negate the possibility of such a mechanism. This longer latent period might merely mean that the substance is not as readily available for secretion as is adrenalin from the adrenals, and the beginning of the response, therefore, is delayed.

The secondary rise may be another manifestation of the phenomenon described by Cannon (1931) in which he concludes that smooth muscle under sympathetic control may be made to liberate a substance with an adrenalin-like action when its motor nerve is stimulated.

d. Peripheral vascular rhythm. Might not the residual secondary rise be a manifestation of a rhythmical vascular response to stimulation? For in some cases there are observed not only two rises, but three (fig. 1D) or even more (figs. 1E and 1F), especially if the stimulation is prolonged. When Carlson (1907) stimulated the cervical sympathetic in the cat, which carries a predominance of vaso-dilator fibers, he observed occasionally a rhythmical variation in the blood flow from the submaxillary gland. This observation would also be in accord with the idea that blood vessel musculature responds to nervous stimulation by rhythmical variations in tone. The heart is a modified blood vessel, and it is not inconceivable that its characteristic property of rhythmical activity is possessed by blood vessels in general.

IV. CONCLUSIONS

1. The secondary rise in blood pressure on peripheral splanchnic stimulation is partly due to the secretion of adrenalin from the adrenals, and partly due to some other factor, for it is seen in modified form after double adrenalectomy.

2. The residual secondary rise is not due to reflexes, contraction of the spleen, or liberation of a pressor or cardio-accelerator substance from the liver, and is probably not due to increased cardiac output. Nor is it the result of any sort of intermittent after-discharge from the stimulated region of nerve, or from the coeliac ganglion.

3. It is believed to be a peripheral phenomenon involving a splanchnic nerve-end organ mechanism. This end organ may be chromaffine tissue liberating an adrenalin-like substance, or some other tissue possibly the blood vessel musculature, elaborating a pressor substance (i.e., Cannon's "sympathin"). Or it may be rhythmically contracting vascular musculature.

The suggestions and advice of Doctor Carlson in this work were invaluable.

BIBLIOGRAPHY

ANREP. 1912. Journ. Physiol., xlv, 307.
CANNON AND RAPPORT. 1922. This Journal, lviii, 308.
CANNON AND URIDIL. 1922. This Journal, lviii, 353.
CANNON AND BACQ. 1931. This Journal, xcvi, 392.
CARLSON. 1907. This Journal, xix, 412.
DREYER. 1898. This Journal, ii, 203.
ELLIOTT. 1912. Journ. Physiol., xliv, 374.
GLEY AND QUINQUAUD. 1913. Compt. rend. Acad. d. Sci., clvii, 66.
1916. Compt. rend. Acad. d. Sci., clxii, 86.
1917. Journ. de Physiol., xvii, 807.
GLEY. 1921. Journ. de Physiol. et Pathol. gen., xix, 355.
1923. Compt. rend. Soc. de Biol., lxxxviii, 1121.
1923. Compt. rend. Soc. de Biol., lxxxviii, 1174.
GUTHRIE AND PIKE. 1907. This Journal, xviii, 14.
HARTMAN. 1923. This Journal, lxiv, 1.
LEHNDORFF. 1908. Arch. f. Physiol., 362.
MELTZER. 1904. This Journal, xi, 28.
PEARLMAN AND VINCENT. 1919. Endocrinol., iii, 121.
STEWART AND ROGOFF. 1916. Journ. Pharm. Exper. Therap., viii, 205.

CENTRAL AND PERIPHERAL LATENCIES IN SOME TENDON REFLEXES OF THE RAT

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Various workers, notably Buchanan (1908), Jolly (1911), Forbes and Gregg (1915), Eccles and Sherrington (1931) and Eccles (1931), have calculated the delay of nerve impulses in the spinal cord subsequent to ipsolateral or contralateral reflex stimulation. All of these workers have used decerebrate or spinal animals. In such preparations only a small part of the nervous system exerts an influence upon the arc or arcs studied. Since it is generally conceded that the most variable factor in reflex time determinations is the state of activity of higher neural levels the above procedure gives no clues to the actual relationships as they exist in the normal animal. Recent work by Lashley and his co-experimenters has definitely denied the validity of atomism in neurophysiology and convincingly pointed to the importance of the entire nervous system in the interpretation of results gained from experimentation on any integral part.

The present paper reports the measured latency in the neurally intact rat's Achilles reflex arc for the receptor, for the spinal cord synapse or synapses, for the myoneural junction and such latency in the spinal cord for the crossed flexion reflex.

APPARATUS AND METHOD. The amplifying-recording unit and the method of reflex elicitation have been described in detail elsewhere (Travis and Dorsey, 1931; Herren and Haterius, 1931). For recording nerve action currents the electrodes consisted of two 2 mm. apart non-polarizable (silver-silver chloride) wires bent to encompass partially the nerve. For recording muscle action currents the electrodes consisted of two 2 mm. apart non-polarizable (silver-silver chloride) wires flattened to rest upon the belly of the muscle.

The animals used were eight mature, white and hooded, male and female, rats. In all of them, under ether anesthesia, the sensory and motor roots of the left fifth spinal nerve, the left internal popliteal nerve and the left gastrocnemius muscle were exposed. Experiments were not undertaken until fifteen to twenty minutes after the anesthesia had been discon-

[1] The authors wish to express their gratitude for the facilities and assistance given them by Professor Lee Edward Travis in whose laboratory this work was done.

tinued. Despite the fact that the anesthetic had been discontinued and the animals allowed to recover (as judged by ability to walk around without staggering and an interest in their surroundings) the animals when placed for recording showed no evidence of discomfort and did not struggle. Records were taken from the sensory and motor roots of the fifth lumbar nerve, from the internal popliteal nerve, and from the gastrocnemius muscle following maximal mechanical stimulation of the left Achilles tendon. From this series of records it was possible to measure all synaptic delays in the ipsilateral reflex. Records were also taken from the left fifth lumbar motor root following stimulation of the right Achilles tendon. From this series of records the delay within the spinal cord was measured for the contralateral reflex. Although electrical stimulation of the fourth lumbar

TABLE 1

The latencies in sigma for the ipsilateral sensory and motor roots, for the internal popliteal nerve and for the gastrocnemius muscle following Achilles reflex stimulation. Also the latency for the contralateral motor root following Achilles reflex elicitation

RAT	LEFT ACHILLES					RIGHT ACHILLES
	Left sensory root	Left motor root	Left internal popliteal		Left gastrocnemius	Left motor root
1	2.3	5.0	1.0	6.2	9.5	6.5
2	2.7	5.1	1.0	7.5	11.5	7.5
3	3.0	5.0	1.0	7.0	10.5	6.5
4	2.9	6.1	1.5	8.0	11.0	8.5
5	2.1	5.6	1.0	7.0	10.5	9.0
6	2.2	5.0	1.0	6.3	10.2	6.5
7	2.6	4.8	1.2	6.5	10.1	6.6
8	3.6	6.8	1.5	9.0	12.3	9.0

motor root showed dorsiflexion of the foot with a weaker induced current than did stimulation of the motor root of the fifth lumbar nerve, preliminary experiments showed sufficient flexion fibres in the latter to give action currents with a latency the same as that in the fourth lumbar nerve.

All times reported herein are the averages of from five to seven consecutive reflex jerks.

RESULTS. In table 1 it will be noted that there are two series of times recorded from the internal popliteal nerve. The shorter of these is interpreted as being the latency of the receptor, hence sensory in nature, and the longer as being the motor discharge. In order to dispel suspicion that this short time might result from interference in our apparatus such as "picking up" the signal circuit (the instant of stimulation is recorded by closing a circuit in which there is a previously charged condenser, (Herren

and Haterius 1931) we discharged this circuit through the animal and found that it was invariably recorded within half a sigma, usually less.

Table 2 shows the latencies of the receptor, the central synapse or synapses, and the myoneural junction of the ipsilateral reflex. This table shows also the central latency of the contralateral reflex. The figures for the latency of the receptor were derived by reading the shorter of the two times recorded from the internal popliteal nerve. The central reflex time was obtained by subtracting the reading off the sensory root from that off the motor root. The latency for the myoneural junction was obtained by subtracting the longer of the two times recorded off the internal popliteal nerve from the time recorded off the gastrocnemius muscle. The crossed central reflex time was obtained by subtracting the time recorded off the left sensory root, following left Achilles tendon stimulation, from

TABLE 2

Latencies in sigma for the receptor, the ipsilateral and contralateral central synapse or synapses, the myoneural junction and the transmission time for motor and sensory sciatic fibers during Achilles reflex elicitation

RAT	LEFT ACHILLES			RIGHT ACHILLES	TRANSMISSION TIME	
	Receptor	Central reflex time	Myoneural junction	Cross central reflex time	Sensory fibers	Motor fibers
1	1.0	2.7	3.3	4.2	1.3	1.2
2	1.0	2.4	4.0	4.8	1.7	2.4
3	1.0	2.0	3.5	3.5	2.0	2.0
4	1.5	3.1	3.0	5.6	1.4	1.9
5	1.0	3.5	3.5	6.9	1.1	1.4
6	1.0	2.8	3.9	4.3	1.2	1.3
7	1.2	2.2	3.6	4.0	1.4	1.7
8	1.5	3.2	3.3	5.4	2.1	2.2

that recorded off the left motor root following right Achilles tendon stimulation. This procedure was considered valid since Herren and Fossler (1931) reported that in the preliminary experiments of their work crossed flexion reflex time in rats was the same on both sides. It is therefore thought that impulses set up by striking either Achilles tendon would be conducted to the spinal cord in the same interval of time. In addition table 2 shows the time required for the transmission of both sensory and motor impulses over the same interval of nervous tissue—the sciatic nerve. The values for the transmission time of the sensory impulses were derived by subtracting the receptor time from the time recorded off the sensory root. Those for the motor transmission time were obtained by subtracting the reading off the motor root from the longest reading recorded off the internal popliteal nerve.

It was singular that regardless of whether the records were taken off the spinal roots or off the internal popliteal nerve the amplitude and duration of the action current flow was always greater in the motor than in the sensory discharge.

Discussion. In all but one case table 2 shows the crossed central reflex time to fall short of twice the ipsolateral central reflex time by a variable figure. Previous workers have found on decerebrate or spinal animals that crossed central reflex time approximates a value twice the ipsolateral central reflex time much more closely than do our results for the same measure. Two factors are probably rseponsible for this discrepancy. The first of these is that in decerebrate or spinal preparations the nórmal inhibitory action of the higher neural levels is removed. This condition would allow an impulse to pass over the arcs involved with no impedance. The second factor is that Herren and Fossler (1931) have recently demonstrated a differential in these two arcs in the inhibition of upper on lower neural levels. They showed that the crossed flexion reflex in rats is normally not under the upper motor neurones, inhibitory control to the same extent as is the ipsolateral extension reflex. These two factors in preparations such as were used in this study would operate to bring about such results as are presented here.

The latency of our myoneural junction is considerably longer than that reported by others. Also our total reflex time is considerably longer (in general about three sigma) than the normal for our mature animals of a similar weight range. However, inasmuch as our animals were very probably partially under the effect of ether at the time of experimentation—it is doubted that twenty minutes is sufficient time for the complete removal from the animal of ether or its effects—and inasmuch as Auer and Meltzer (1914) have reported a "curare like action" for ether, the longer times for these latencies can be understood.

It is interesting to note that an equal time is taken for transmission of the afferent and efferent impulses over the same interval of the same nerve trunk. Contrary to our results Eccles and Sherrington (1931) working on spinal cats have recently reported that the motor fibers conduct from two to three times faster than the sensory fibers in reflex dorsiflexion of the foot. We cannot explain this discrepancy but call attention to the difference between the preparations used in the two studies and also to the fact that they elicited reflexes with induction shocks to the nerve trunk.

The fact that the motor action currents are of greater amplitude and of longer duration than the sensory ones indicates an amplification process within the central nervous system for incoming impulses. This amplification may be conceived as indicating that any one sensory fiber must functionally communicate with several motor fibers Such a relationship has anatomical evidence for its support. On the other hand it is univer-

sally conceded that the most complex station in any reflex arc is within the nerve center and that at this point the impressed sensory impulses are subjected to the influence of the existing nervous equilibrium (Travis and Dorsey 1929a, b; 1930), (Herren and Haterius, 1931), which could bring about this amplification. Again, this phenomenon may be reconciled with a growing conviction that the sheer mass of responsive tissue is an important factor in any nervous function (Lashley, 1929). The sensory side of the arc may be conceived as consisting of only the receptors and the posterior horn cells and their processes. The motor side of the arc however may consist not only of the anterior horn cells and their processes but the entire remainder of the central nervous system. This explanation draws support from recent work which demonstrates that all parts of the cortex (Travis and Herren, 1931) and probably the cord (Travis and Fossler, 1931) are involved in reflex activity.

SUMMARY

1. The range for the receptor latency of the Achilles tendon reflex in the rat is from 0.001 to 0.0015 second.

2. The range for the latency of the central reflex time for the same reflex is from 0.002 to 0.0035 second.

3. The range for the latency of the crossed central reflex time is from 0.004 to 0.0069 second.

4. The range of latency for the myoneural junction in the Achilles tendon reflex is from 0.003 to 0.0039 second. This is believed to be slightly exaggerated due to the anesthetic used.

5. The transmission time for sensory fibers is about equivalent to the transmission time for motor fibers for the same interval of nerve tissue.

BIBLIOGRAPHY

AUER AND MELTZER 1914. Journ. Pharm. Exper. Therap., v, 521.
BUCHANAN. 1908. Quart. Journ. Exper. Physiol., i, 1.
ECCLES AND SHERRINGTON. 1931. Proc. Royal Soc., B, cxvii, 511.
ECCLES. 1931. Proc. Royal Soc., B, cvii, 557.
FORBES AND GREGG. 1915. This Journal, xxxvii, 118.
HERREN AND HATERIUS. 1931. This Journal, xcvi, 214.
HERREN AND FOSSLER. 1931. This Journal, xcvii, 282.
JOLLY. 1911. Quart. Journ. Exper. Physiol., iv, 67.
LASHLEY. 1929. Brain mechanisms and intelligence. Univ. Chicago Press.
TRAVIS AND DORSEY. 1929a. Arch. Neurol. and Psychiat., xxi, 613.
1929b. Ibid., xxii, 99.
1930. Arch. Neurol. and Psychiat., xxiv, 48.
1931. Arch. Neurol. and Psychiat., in press.
TRAVIS AND FOSSLER. 1931. Proc. Soc. Exper. Biol. and Med., xxviii, 1043.
TRAVIS AND HERREN. 1931. Journ. Comp. Psychol., xxii, 23.

OSMOTIC RELATIONS OF BLOOD AND GLANDULAR SECRETIONS

I. The Regulatory Action of Total Blood Electrolytes on the Concentration of Gastric Chlorides[1]

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The means by which the stomach glands regulate the concentration of chloride in the secretion which they elaborate is yet to be explained satisfactorily on an experimental basis. Furthermore, all investigators are not in accord concerning the fundamental fact as to the normal concentration of chloride in the gastric juice. One group of workers, making observations on human subjects, are of the opinion that the level of gastric chloride content is essentially the same as that of blood chloride, namely, approximately 110 milli-equivalents—*m.eq.* (MacLean and Griffiths, 1928; Apperly, 1925; Bulger and Allen, 1927; Bulger, Allen and Harrison, 1928; Bulger, Stroud and Heidemann, 1928). There are those who believe that much higher values for the concentration of gastric chlorides occur—114 to 171 *m.eq.*—but that the ionic content of the gastric juice as found in the stomach is fairly characteristic for the individual although it may be constant as it leaves the glands (Polland, Roberts and Bloomfield, 1928). Lastly there are studies unanimous in their conclusion that the concentration of gastric chloride is approximately 160 *m.eq.* The fact that such a concentration of electrolyte is approximately the same as that observed in the blood has been regarded as indicating a causal relationship between the ionic content of these two fluids (Gamble and McIver, 1926, 1928; Berglund, Walquist and Sherwood, 1927; Hollander, 1928; Hollander and Cowgill, 1931).

If appreciable changes in the ionic content of the blood could be produced experimentally, it would follow from the last theory stated above that the ionic content of the gastric juice would exhibit parallel changes. Such observations would indicate the true concentration of chloride in the gastric

[1] The data in this paper are taken from a dissertation presented by Alfred Gilman to the Faculty of the Graduate School of Yale University in partial fulfillment for the degree of Doctor of Philosophy, 1931.

A preliminary report of this paper was presented to the American Physiological Society at Montreal, April, 1931.

juice and the means by which this level is maintained. This paper is a report of such experiments.

EXPERIMENTAL PROCEDURE. Two dogs, one (A) possessing a Pavlov fundic pouch, and the other (B) a Heidenhain fundic pouch, were used in this study. Variation in the concentration of blood electrolytes was produced by dehydration. It was found that, by depriving the animals of water for a period of three days, it was possible to produce a significant rise in the ionic content of the blood. When the dogs were allowed to drink, this value returned to normal.

Gastric juice was obtained subsequent to histamine stimulation. As has been previously reported (Babkin, 1931; Gilman and Cowgill, 1931) this drug acts exclusively on those glands concerned with the elaboration of the chlorides of the gastric secretion. Subsequent to histamine injection, with increasing volume of secretion, there is an increase in acidity of the gastric juice. The total chloride content, however, remains relatively constant. When the rate of secretion and acidity reach their maxima, practically all of the chloride of the stomach secretion is present as HCl, the concentration of both the basic and enzyme constituents of this fluid being at a minimum. The gastric juice obtained under these conditions was regarded, therefore, as being the truest representation of the chloride secretion of the stomach, and was used as a basis of comparison in the various experiments.

The gastric juice was analyzed for total and acid Cl. Chloride represents practically all the anion of this fluid. Furthermore it is present in dilute solution, either as a strong acid (HCl) or as the salt of a strong base (NaCl). Consequently it may be considered to be completely dissociated. Thus the concentration of the total chloride of the gastric juice not only represents the total ionic content of this fluid, but it also bears a direct relationship to its osmotic pressure. Likewise the concentration of the fixed base of the blood is indicative of the total ionic content and osmotic pressure of that fluid (Gamble, 1923); consequently the examination of the blood was confined to a determination of this variable. Analyses were made on the serum, the method of Stadie and Ross (1925) being employed.

Gastric juice was collected from the animals when in a normally hydrated condition. Coincident with the collection of this juice, a sample of blood was drawn from the jugular vein. The dogs were then dehydrated and the experiment was repeated. Successive hydrations and dehydrations were performed until the results seemed conclusive.

EXPERIMENTAL RESULTS. The results from dog A are illustrated in figure 1. As can be seen from this graph, an increase in the concentration of blood electrolytes resulting from the restricted water intake led to an equal and parallel increase in the concentration of chloride in the gastric secretion. For example, period 2 represents a time during which the ani-

mal was normally hydrated. At the conclusion of this period the concentrations of gastric chloride and blood base were approximately normal,

Figure 1

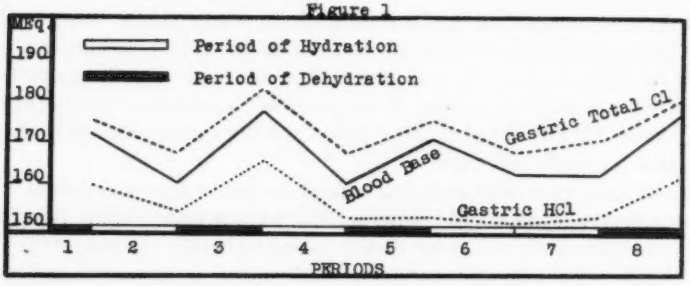


Figure 2

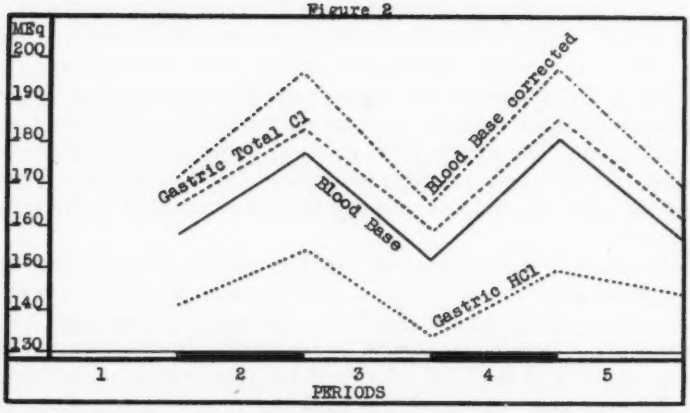
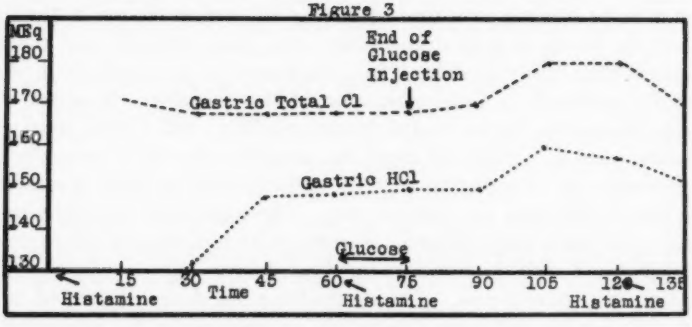


Figure 3



168.0 and 161.0 m.eq. respectively. Subsequent to this the dog was dehydrated. The results of this procedure led to an increase in the total base concentration of the blood, the significantly high figure of 178.0 m.eq. being

obtained. At the same time the concentration of gastric chloride rose to 183.2 m.eq. Subsequent hydrations and dehydrations led to successive decreases and increases in the levels of both blood base and gastric chlorides as shown in the graph.

The figures obtained from the analyses of these two fluids show the gastric juice to have a higher ionic content than the blood. This would imply that the first-named fluid is hypertonic. However the values representing the concentration of base are expressed as *m.eq.* per liter of serum. A much more accurate conception of the true ionic composition of this fluid can be obtained if one determines the total solid content of the serum and corrects for this value. The results would be an expression of the concentration of fixed base per liter of water. Such a figure would be much more accurate in comparison with the gastric juice which has a negligible content of solids. The results of such a calculation are illustrated in figure 2 (dog B).

In figure 2 we see, as before that an increase in the electrolyte content of the blood leads to an equal and parallel increase in the total ionic content of the gastric juice. However, the value for the total base of the blood serum corrected for total solids is higher than the value expressing the concentration of gastric chloride, the gastric value falling between the corrected and uncorrected blood figures.

From the above experiments, it is impossible to state whether the blood and gastric juice are isotonic. A positive assertion in this regard must be reserved until accurate measurements of the respective osmotic pressures of these two fluids can be made; investigations designed to yield such data are now in progress. However, it is difficult to see how the above results can be interpreted in any other way than to imply that some osmotic relationship exists between blood and gastric juice, the total electrolyte concentration of the former being the influential factor in determining the concentration of ions of the latter. Inasmuch as chloride represents practically all of the anion of the gastric juice the above statement implies that the chloride concentration of the stomach secretion is regulated by the osmotic pressure of the blood.

Discussion. Assuming the above statement to be correct, it is interesting to speculate as to the mechanism by which this osmotic equilibrium is maintained. If it be granted that the concentration of chloride in the gastric juice is such as to make this fluid approximately isotonic with the blood, it must of necessity follow *that the gastric mucosa and cells of the gastric glands are impermeable to chloride ion.* If chloride were able to freely diffuse in either direction between the blood and gastric juice, then this ion would distribute itself so that its concentration in the two fluids would be the same. If H, OH and Cl were the only permeable ions and one assumed a Donnan equilibrium to exist between the blood and gastric juice, the level of chloride

in the stomach would be still less than that of the blood. Obviously neither of these conditions exists. It must therefore be assumed that the gastric walls are impermeable to this ion, the presence of chloride in the stomach juice being the result of an active secretory process of the parietal cells. If chloride, once transported across the cell walls into the lumen of the gastric glands is unable to return, then water would diffuse in either direction across the cell membrane until the fluids become isotonic. Such a diffusion might possibly take place in the lumen of the glands or as the juice flows over the mucosa, the former theory appearing to be the more logical. If chloride is secreted in dilute solution, then there would be an absorption of fluid as the juice passed into the stomach proper. Observations of an increase in acidity of the gastric juice as it passes down the glandular lumen support this view, the increase in acidity according to the above theory being due to water absorption from, and consequent concentration of the acid secretion. Dawson and Ivy (1926) offered such a suggestion to explain the differences which they observed between the staining reactions of the parietal cells and the fluid at the crypt of the gland. The experiments presented here are interpreted as additional evidence that such a water exchange takes place.

This theory can be tested in another way. If the concentration of gastric chloride is determined by a water exchange between the blood and gastric juice, then any agent causing an increase in the osmotic pressure of the blood should bring about a corresponding increase in the osmotic pressure of the stomach secretion. In the experiments cited above, such an increase in the osmotic pressure of the blood was accomplished by dehydration. It also must follow that any substance introduced into the blood stream in high enough concentration to increase the osmotic pressure of that fluid should cause an increase in the chloride concentration of the gastric juice. Thus even such a substance as glucose, which, although not an electrolyte, exerts an osmotic pressure proportional to the number of molecules in solution, if present in the blood stream in sufficient concentration, should bring about a rise in the level of gastric chlorides.

An opportunity to test this idea arose when it became necessary to sacrifice a dog possessing a gastric pouch. The animal under amytal anesthesia was made to secrete in response to a histamine stimulus, this drug being injected at hourly intervals. When the concentration of the total and acid chloride of the gastric juice had become constant and characteristic, 100 cc. of a 50 per cent glucose solution were injected into the femoral vein. The result of the injection is shown in figure 3. After a latent period of 15 minutes, there was observed an appreciable rise in the concentration of both total Cl and HCl. This lasted for 30 minutes after which the concentrations became normal and characteristic. It can be seen that the results of this experiment also confirm the working hypothesis.

In view of these confirmations it follows that there is a definite limitation with respect to the maximum acidity that the gastric juice is able to attain, namely, when practically all the Cl is present as HCl. That this maximum acidity, about 160 *m.eq.*, has been repeatedly observed in various species of animals (see Hollander and Cowgill's (1931) review of literature bearing on this) may be cited as further evidence in favor of this view; and the fact that it has never been exceeded, taken in conjunction with the experiments reported in this paper, indicates that the osmotic pressure of the blood is the limiting factor determining the chloride concentration and the minimum pH of the gastric secretion. From this point of view it is unnecessary to imagine the secretion of a neutral chloride as an essential feature in limiting the acidity of the gastric juice, an idea that has been advanced by MacLean and Griffiths (1928).

The low values for gastric chlorides that have been observed by those investigators working with human subjects can doubtless be explained by contamination of the gastric contents with test meals and body secretions other than gastric juice. Dilution from the other glandular secretions of the gastric mucosa may play a very slight rôle. However, it is significant that in view of the physico-chemical considerations presented above, pure gastric juice should have a chloride content of approximately 160 m.eq.

SUMMARY

Experiments have been presented to show that the total chloride content of pure gastric juice obtained in response to a histamine stimulus from dogs possessing gastric fundic pouches is approximately equal to the total ionic content of the blood. Variations in the total concentration of blood electrolytes, produced by alternate dehydration and hydration of the animal, were associated with parallel changes in the chloride content of the gastric secretion. Physico-chemical considerations were advanced to support the view that the process by which the chloride concentration of the gastric juice is maintained depends upon a water exchange between a chloride solution in the stomach and a blood of given total ionic content.

BIBLIOGRAPHY

APPERLY, F. L. 1925. Med. Journ. of Australia, June.

BERGLUND, H., H. WALQUIST AND K. SHERWOOD. 1927. Proc. Soc. Exp. Biol. and Med., xxiv, 927.

BULGER, H. A. AND D. S. ALLEN. 1927. Proc. Soc. Exp. Biol. and Med., xxiv, 902.

BULGER, H. A., D. S. ALLEN AND L. B. HARRISON. 1928. Journ. Clin. Inves., v, 547.

BULGER, H. A., C. M. STROUD AND M. L. HEIDEMANN. 1928. Journ. Clin. Inves., v, 547.

DAWSON, A. B. AND A. C. IVY. 1926. This Journal, lxxvi, 158.

GAMBLE, J. L. AND M. A. MCIVER. 1926. Proc. Soc. Exp. Biol. and Med., xxiii, 439. 1928. Journ. Exp. Med., xlviii, 837.

GAMBLE, J. L., G. S. ROSS AND F. F. TISDALL. 1923. Journ. Biol. Chem., lvii, 633.
GILMAN, A. AND G. R. COWGILL. 1931. This Journal, xcvii, 124.
HOLLANDER, F. 1928. Proc. Soc. Exp. Biol. and Med., xxv, 486.
HOLLANDER, F. AND G. R. COWGILL. 1931. Journ. Biol. Chem., xci, 151.
MACLEAN, H. AND W. J. GRIFFITHS. 1928. Journ. Physiol., lxv, 63.
POLLAND, W. S., A. M. ROBERTS AND A. L. BLOOMFIELD. 1928. Journ. Clin. Inves., v, 611.
STADIE, W. C. AND E. C. ROSS. 1925. Journ. Biol. Chem., lxv, 735.
VINEBERG, A. M. AND B. P. BABKIN. 1931. This Journal, xcvii, 69.

INQUIRIES INTO THE STRUCTURE AND FUNCTION OF THE MYENTERIC PLEXUS

I. Differences in the Reaction of the Muscle and Nerves of the Bowel to Constant and Interrupted Currents

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While studying conduction in the bowel of the rabbit (Alvarez and Hosoi, 1930) marked differences were noted in the responses of the wall of the gut to faradic and galvanic stimuli, differences which seemed to conceal some secret worth knowing. The principal facts observed were: first, that with the constant galvanic current the curve of contraction was simple, and conduction along the bowel was absent, and, second, that with the faradic tetanic current the curve of contraction was composed of two elements, and conduction of the disturbance along the bowel could be detected.

The difference in the shape of the curves will be seen from figure 1. (The technic of preparing and anesthetizing the animals and using excised segments has been described by Alvarez and Hosoi, 1929.) When a current of 1 or 2 milliamperes was allowed to flow for a second through specially designed platinum electrodes touching the mesenteric edge of the bowel, the lever attached by a thread to the antimesenteric border of the stimulated segment almost always rose sharply; the latent period was about 0.15 second, the amplitude of contraction was small, and relaxation was usually prompt and complete (fig. 1, d). Sometimes a spontaneous contraction would interrupt the fall of the lever to the original baseline.

With a tetanizing current obtained from a Porter inductorium energized with one dry cell and with the secondary coil at 4 cm., there was first a latent period of approximately twenty-five hundreth second; then the writing point of the lever rose slowly for about a second, and after that it shot up quickly (fig. 1, a, b, g, m). The amplitude was usually greater than that seen with galvanic stimulation and the period of relaxation was longer.

[1] Aided by a grant from the Josiah Macy, Jr. Foundation.

Rarely, as in figure 1 g, faradic stimulation produced a little "hump" before the second rise due to the fact that the muscle after the first stimulus was beginning to relax before it contracted again.

Occasionally, with the faradic current, the first contraction was so quick that the curve resembled that obtained with galvanic stimulation (fig. 1, k) but ordinarily the two types of contraction could be distinguished at a

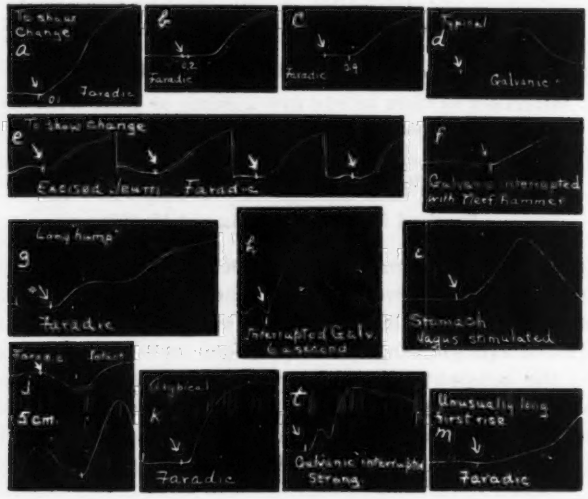

Fig. 1, *a*. A faradic response with a quick, steep first rise. *b*. The same type of curve with the first rise barely perceptible. In curve *c*, if the muscle contracted it did not succeed in lifting the writing point of the lever and the latent period became that of the second rise. *d*. Galvanic response to the closing of the galvanic current. *e*. With successive faradic stimuli the curve of contraction changed from galvanic to faradic. *f*. An unusually good response to galvanic current interrupted with a Neef hammer. *g*. An unusually pronounced first faradic contraction. *h*. The usual response to a slowly interrupted galvanic current. *i*. Composite curve of contraction of gastric muscle when one vagus nerve was stimulated with a tetanizing faradic current. *j*. Local relaxation with contraction 5 cm. caudad to the segment stimulated. *k*. Galvanic response to a faradic stimulus. *t*. Preliminary "hump" obtained with a strong interrupted galvanic current. *m*. Unusually long first rise with a faradic stimulus.

glance. Sometimes when, with the first faradic stimulus thrown in, the lever rose sharply, as in figure 1 a, with subsequent stimuli it rose more and more slowly, as in figure 1 b, until the first shortening of the muscle failed to lift the lever and the latent period then became that of the second contraction, as in figure 1 c. Figure 1, a, b, c, and e, show this transition from one type of response to the other.

In the intact animal the interval between the throwing in of the stimulus and the beginning of the steep rise in the faradic records varied from 0.5 to 2.1 seconds. When 134 measurements from all parts of the small bowel were arranged in a distribution polygon there was a decided mode at 1.4 seconds. The mode for fifty-nine similar measurements made on excised bowel was 1.2 seconds. The interval was a little longer in the lower part of the ileum than it was elsewhere in the small bowel.

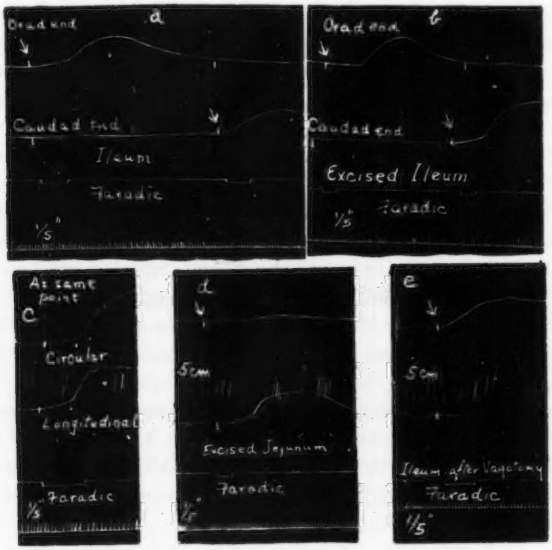

Fig. 2. In this and in all subsequent tracings, an arrow marks the point and moment of stimulation. The figures between the curves denote distances between the attachment of recording threads on the gut. *a* and *b*. Different types of response to faradic stimulation at the orad and caudad ends of a loop of excised rabbit ileum 5 cm. long. *c*. Simultaneous contractions of circular and longitudinal muscle in the same narrow segment of gut stimulated with a tetanizing current. *d*. The response more prompt and with greater amplitude at a short distance from the point stimulated. *e*. Local contraction of galvanic type with the distant response also galvanic.

We might not have spent so much time in an effort to explain these differences if, at the beginning, we had seen how often, especially in excised bowel, the faradic stimulus will produce a galvanic type of reaction, or if we had seen how the response can vary from moment to moment, or at two points 5 cm. apart on the same loop of bowel (fig. 2, a and b). We might have become discouraged or might have decided that the difference was more or less accidental. In the intact bowel, a galvanic type of response to a faradic tetanizing stimulus was observed in perhaps 9 per cent of the ex-

periments. It was observed with 12 per cent of the stimuli thrown into the duodenum, 16 per cent in the jejunum, 5 per cent in the upper part of the ileum, and 4 per cent in the lower part of the ileum. It was observed much more commonly with excised bowel and particularly with excised duodenum. Again, there was a gradation from one end of the bowel to the other, the percentage of galvanic responses to a faradic stimulus being for duodenum, jejunum, upper ileum, and lower ileum, 60, 27, 24, and 19, respectively. Taking the 454 experiments on all parts of the excised bowel together, the percentage was 34. It is probable that in some instances we were mistaken, and what looked like galvanic responses were of the type depicted in figure 1 c. The response to the constant galvanic current was almost always a contraction with the simple type of curve depicted in figure 1 d.

Our impression is that the greater the irritability of a segment of bowel the more likely it is to respond quickly with a sharp rise of the lever. Excised bowel is more responsive to stimuli than is intact bowel, perhaps because inhibition has been removed; and as Alvarez and Hosoi (1929) have shown, there is in the bowel of the intact animal, a gradient of irritability from duodenum to ileum. The diminishing tendency of the muscle in successive regions of the bowel to respond in a quick galvanic way to a faradic stimulus is therefore probably just another manifestation of the gradient of irritability.

Although the lack of specificity of the two types of reaction complicates the problem and to some extent makes it seem less worthy of study, we feel that the efforts we have made to solve it, although not entirely successful, have been well worth while if only because there have been so many interesting by-products.

Differences in the behavior of circular and longitudinal muscle. One of the first suggestions that occurred to us was that the composite nature of the curves produced with a faradic stimulus might be due to separate contractions of the fibers in the two muscular coats of the bowel. Accordingly several attempts were made to record simultaneously, with threads attached to levers, the contractions of these two coats. Unfortunately the attachment of so much apparatus to a short segment of bowel seemed to handicap it so much that usually the latent periods were long and the contraction curves unsatisfactory. Occasionally both coats did contract promptly, with the longitudinal generally a little ahead of the circular (fig. 2 c). Sometimes the two coats shortened together for a few tenths of a second and then the marked contraction of the circular fibers brought about a lengthening of the longitudinal ones. At other times the two coats continued to contract together.

In order to get rid of the irritation and drag produced by mechanical recorders we next made four black marks on the surface of the gut; we put the

electric switch and a split second watch into the field of a motion picture camera and made a number of films showing the responses of the two coats.

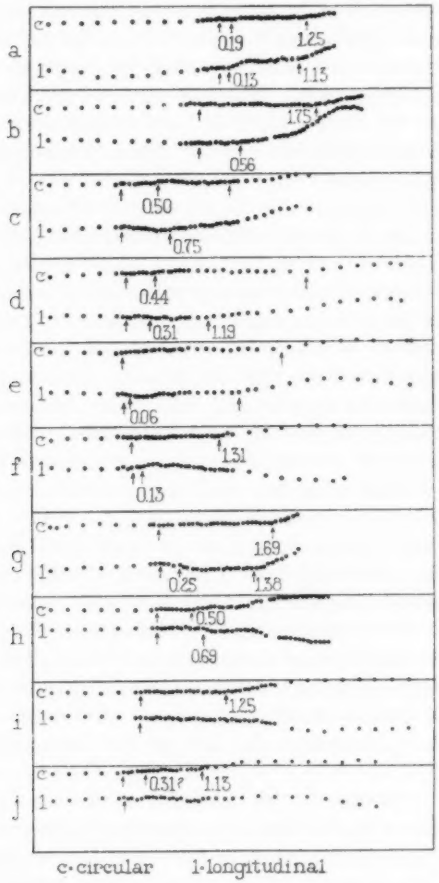

Fig. 3. Curves obtained by measuring in successive frames of a motion picture film the distances between two sets of points on a small segment of small intestine. The distances were plotted as ordinates with intervals of time as abscissas. In each section the upper curve represents contraction of the circular muscle, the lower curve of the longitudinal muscle. The first arrow shows the moment of stimulation; subsequent arrows show beginning of contraction. Figures represent seconds elapsed after the stimulus.

Successive frames were projected onto a screen and the distances between the markers were measured and plotted as ordinates with intervals of time as abscissas.

The results would have been more satisfactory if the muscle had responded more often with a short latent period. Possibly it would have done so if it had been stretched slightly as it is when a writing lever is attached. The first record in figure 3 shows that the longitudinal muscle began to contract slightly after 0.13 second and more markedly after 1.13 seconds. The circular coat contracted slightly after 0.19 second and more markedly after 1.25 seconds. The fact that the first component of the curve had about the same length in both records showed that the composite nature of the faradic curve is not due to differences in the reaction of the two sheets of muscle.

In the next four curves it may be noted that although the longitudinal muscle contracted first, some time before the circular, this shortening did not affect the length of the circular fibers. There are many statements in the literature to the effect that contraction in one coat produces lengthening in the other, but this is not always true, and when it appears to be so an analysis of the record will sometimes show that the lengthening of one muscle layer began before the other shortened. This is apparent in figure 3, f and g. There the longitudinal coat, after temporary contraction, steadily relaxed long before it could have been forced to do so by a contraction of the circular coat. In records made with levers we found a few such curves in which we had to measure the latent period to a point of relaxation instead of to a point of contraction (fig. 1, j).

In figure 3, h and i, the relaxation of the longitudinal coat appears to be due to contraction of the circular. In figure 3, j, both coats continued for almost three seconds to contract together, and then the longitudinal muscle relaxed. We have other curves plotted from photographic records of loops of bowel contracting spontaneously which show again that the activities of the two muscular layers are often independent and that much of what has been written on this subject must be revised. As yet we have no explanation for the fact that they act one way one moment and another way the next.

Other possible explanations. It seemed possible that the first rise after stimulation might at times represent a spontaneous rhythmic contraction with an artificial one superinduced, and this may be the explanation in rare instances (fig. 1, g). That it is not the explanation in most cases was indicated by the fact that the curve was composite in all those records made with segments of bowel that for some time had shown little if any tendency to spontaneous rhythmic activity.

In some records there was a suggestion that the second rise might be due to the final interruption of the stimulating current, but in many others it was obvious that this could not be the cause. Another thought was that there might be two types of muscle fiber in the wall of the bowel, or two types of contractile substance with different strengths and different rates

of shortening, and this is still a possibility; we have not yet found a way of proving or disproving it. It is conceivable also that the tetanizing current might act first on some inhibiting mechanism in the wall of the bowel before it produces contraction. Occasionally the bowel did respond first with relaxation, especially when the electrode had slipped so that perhaps it stimulated nerves in the mesentery (fig. 1, j).

When "humps" appeared in records of contraction following a single galvanic stimulus they came late, and apparently represented spontaneous rhythmic waves. It does not seem probable that the galvanic current stimulates the bowel more than does the faradic because the sharp rise of the lever with the former was seen even with the weakest intensities; and with the strongest, the amplitude of contraction was never so great as that seen with faradic stimulation.

We thought we might perhaps steepen the initial slow rise of the lever, which is so characteristic of the response to the tetanizing current, by increasing the intensity of the stimulus, but even with 45 volts in the primary circuit and the secondary coil at 0 cm., no change was observed in the shape of the contraction curve.

As already stated, at times records were obtained in which the curve of contraction after faradic stimulation rose just as abruptly as it did after galvanic stimulation, but even then, after about a second, a change could often be detected in the rate of acceleration (fig. 1, a).

Interrupted galvanic stimulation. It next occurred to us that the second rise in the faradic curves might be due to a summation of stimuli. In this case we should be able to produce it by interrupting the galvanic current. We tried first breaking it rapidly with the hammer of the inductorium so that the main difference between the galvanic and faradic stimuli would be one of voltage. The results obtained were generally poor. Often there was no response and when one appeared the amplitude was usually so low that we could not be sure of the presence or absence of a second rise; in several instances, however, there appeared to be one. In figure 1, f, which represents the best contraction obtained, the curve is simple. In the many experiments performed, conduction, or the spread orad and caudad of some disturbance produced by the stimulus, could not be demonstrated.

We next interrupted the current more slowly, and when the rate dropped below fifteen a second we began to get good responses locally and at a distance. The best results were obtained with a rate of six a second. Stimulation at this rate gave excellent responses and good signs of conduction; both better at times than those obtained with faradic stimulation. The latent period was short and the amplitude was sometimes even greater than that usually obtained with the faradic stimulus. With a rapid rate of interruption an increase in the strength of the current, even up to 60 ma., did not give better results. Our conclusion was that the greater amplitude

of the response to the tetanic faradic current compared with that to the constant galvanic current is the result of summation.

In the curves obtained with slowly interrupted galvanic stimuli there was seldom any sign of a second rise; that is, almost all of the curves of contraction were of the galvanic type (fig. 1, h). The reason for this may be that the first rise was so steep that when the second contraction came there was no difference in the rate of acceleration and hence no break in the curve. There appears to be something about the volume of the low voltage current which, even when it is interrupted, causes the muscle to contract more quickly and efficiently at first than it does with the quickly changing high voltage current. Figure 1, t, shows that with a strong interrupted galvanic current there may be a marked preliminary hump; relaxation came late, as it does with the faradic current.

The inability of the intestinal muscle to respond well to a rapidly interrupted galvanic current would seem at first glance to be easily explainable on the assumption, made by several investigators, that the tissue is too sluggish and its chronaxie too large. Against this theory is the fact that the bowel responds well to the even more rapidly interrupted faradic current. We tried the effect of strong faradic single make-or-break shocks (with 45 volts in the primary circuit) but the bowel often failed to respond and when it did contract, the amplitude was so low that nothing could be determined about the nature of the curve.

Condenser discharges. We next tried the effect of powerful condenser discharges. Although some of these were of very short duration there was a definite response, with a low galvanic type of curve. This success in obtaining contractions with such fleeting stimuli made it seem probable that the failure with single faradic shocks was due, not to the short duration of the stimulus, but to an insufficient input of energy.

Stimulation of muscle and nerve. Early in this study it occurred to us that the first rise in the curve of contraction might be due to a direct stimulation of the muscle by the current and the second rise to an indirect effect through the myenteric nerves, and particularly through nerves interrupted by a synapse. It is easy to imagine a constant current exerting a greater effect on a sluggish tissue such as smooth muscle than on a more quickly acting tissue such as a nerve.

As we pointed out in a previous paper (Alvarez and Hosoi, 1930) the tetanizing faradic current, when applied to the wall of the bowel, usually produces a disturbance which can be detected from 5 to 15 cm. orad and from 5 to 20 cm. caudad from the point stimulated. The constant galvanic current similarly applied to the bowel rarely produces a disturbance which can be detected more than 3 cm. orad or 5 cm. caudad from the point stimulated. The fact that the contraction at a point 5 or 10 cm. distant from the place stimulated often appears before the muscle has contracted locally

(fig. 4, b, c, and d), or even while it is relaxing (fig. 1, g), would alone show that the disturbance must travel by way of nerves and not as a wave of contraction along the muscle sheet, and there are a number of other observations pointing to the same conclusion. Thus it is a curious fact that the contractions which are recorded at points 5 and 10 cm. caudad to the point stimulated are commonly synchronous, and occasionally, as in figure 4 d, the muscle 10 cm. distant contracts before the muscle 5 cm. distant. In

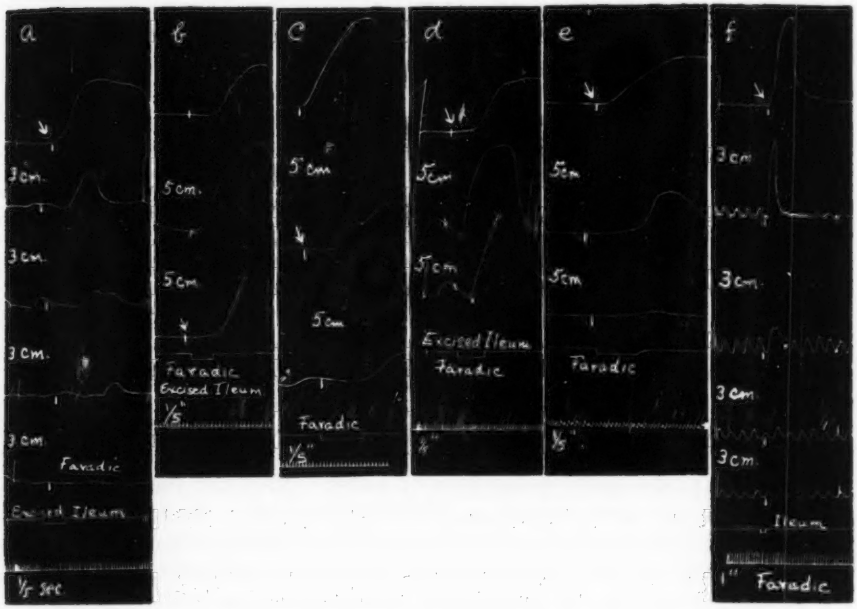

Fig. 4. *a* and *f* show conduction with a decrement. In *b*, *c*, and *d*, a contraction appeared more promptly at a distance than at the point stimulated. In *d* the contraction 10 cm. distant appeared shortly before the contraction 5 cm. distant from the point stimulated. In *c* the local response to a faradic current was galvanic in type, while the response 5 cm. caudad was faradic in type; an unusual record.

most of the records it appeared that the stimulus travelled slowly for the first 5 cm. and then rapidly for the next 5 cm.

More evidence for a neurogenic type of conduction is to be found in the fact, to be reported later in this paper, that after the death of a rabbit the ability of the bowel to conduct is lost long before the muscle loses its ability to respond to a direct stimulus. As one would expect, also, we found conduction to be stopped by the scar made by cutting through the muscle

to the mucous membrane and allowing the wound to heal. As Meek showed years ago, peristaltic rushes can easily jump over such a scar, but the reason for this is obvious from the studies of Alvarez. Further evidence of a nervous conducting mechanism has been presented by Fleisch and Wyss who found that the wave-like disturbance produced by a stimulus would pass through a segment of bowel held firmly in a clamp. They noticed also, while analyzing the movements of the bowel as depicted in motion pictures, that the wave of contraction occasionally seemed to skip short segments where there was no apparent shortening of the muscle.

Extrinsic nerves of the bowel are largely unresponsive to single stimuli. If conduction is due to nerves, as it almost certainly must be, it follows that the constant galvanic, the rapidly interrupted galvanic, and the single faradic stimuli which usually do not produce a disturbance at a distance from the point stimulated do not affect the myenteric nerves. If this is true, one would expect to find that the extrinsic nerves of the bowel also fail to respond to these types of stimuli, and actually, as Lapicque has pointed out, the vagus, like some of the other autonomic nerves is "iteratif," that is, it responds only to repeated stimuli. This fact does not seem to be generally known, but it has been commented on by a number of experimenters. Thus, Rogers and Bercovitz, using the turtle, found that the continuous passage of a galvanic current, or single induced shocks, even of great strength, were without effect on the vagus. (See also Klee, p. 576.)

Our efforts to confirm these observations were made difficult by the fact that in the rabbit it is often impossible, even with a tetanizing stimulus to the vagus to elicit any response from the bowel. Often, too, the bowel will respond at one point and not at several adjacent ones. So far, in many experiments, we have obtained only one record showing a slight response of the stomach to a single galvanic stimulus applied to one vagus nerve, and we have never seen any effect on the bowel of single galvanic or faradic shocks applied to the vagus, the splanchnic, or the mesenteric nerves. According to Miller and Waud, in the decapitated cat, single shocks applied to the mesenteric nerves will produce reflex contractions of the abdominal muscles.

For all practical purposes it would seem that a single stimulus applied to the extrinsic nerves of the bowel has no effect. The next question is: Why, in our experiments, did the extrinsic and intrinsic nerves of the bowel fail to respond well to galvanic stimuli rapidly interrupted? There was a good response with six interruptions a second, but not with fifteen a second. This is hard to understand in the face of the fact that there was so good a response to the much more rapidly interrupted faradic current. Furthermore, Lapicque and Meyerson, Melik-Megrabow, Veach, and others found that the stomach in a number of animals responded when the stimulus

applied to the vagus nerve was interrupted as many as 250 times a second. This was to be expected from the fact that even in the sluggish frog the chronaxie of the vagus nerve appears to be from 2 to 10 sigma.

Figure 1, i, shows that after stimulation of a vagus nerve near the cardia the curve of contraction of the gastric muscle will sometimes show a small preliminary hump. Single shocks applied to the mesenteric nerve had no effect on the bowel.

Latent period of the effect of stimulation of the vagus. If the second rise in the curve of contraction were due to an impulse arriving by way of two neurones connected by a synapse, one would expect to find a long latent period, and since there are reasons to believe that there are synapses between the vagus fibers and those of Auerbach's plexus, an idea might be obtained of what this latent period should be by stimulating the bowel through the vagus nerves. Actually, when we stimulated the vagal trunks immediately below the diaphragm we found that the latent period of the first effect (contraction or relaxation) produced in the bowel was almost the same as that of the second rise in the curve obtained when the bowel was stimulated directly. The range of 57 measurements of the latent period of the vagus effect (with a tetanizing faradic stimulus) was from 0.4 to 3.2 seconds, with the mode around 1.1 seconds. With an interrupted galvanic stimulus (rate, six times a second) the mode was about 1.5 seconds. The mode for the latent period of the second rise (with the stimulus direct to the bowel) was 1.4 seconds.

In a few instances the graphic record indicated that stimulation of the vagal trunks had an almost immediate effect on the bowel, due perhaps to the presence of a few nerve fibers running directly to the muscle. Figure 1, i, shows such a record, in which the muscle of the stomach contracted first after 0.2 second and later after 1 second, just as it might have done if the electrodes had been applied directly to the wall of the viscus.

Although some of these observations suggest strongly that the second rise is due to a stimulus arriving by way of nerves, other observations, to be described later, make it doubtful if this is the explanation.

Attempts to separate the effects of stimulation of nerve from those of stimulation of muscle. If, when the intestine is stimulated directly, the second rise were due to an impulse arriving by way of the myenteric nerves (which we have shown to conduct caudad more easily than orad) it might be possible to remove these impulses and thereby to simplify the curve of contraction by cutting the bowel across just orad to the segment being stimulated. Actually a study of a large number of records made from short excised segments of bowel showed that when, as not infrequently happened, the curves of contraction obtained with a tetanizing current were different at the two ends, the curve at the orad end was more often of the faradic type and that at the caudad end was of the galvanic type (fig. 2, a). The

explanation may be that influences descending from the orad cut end caused the neuromuscular mechanism near-by to suffer from trauma and to become less sensitive. Usually the threshold was lower and the amplitude of contraction greater at the caudad than at the orad end, and often the irritability (response to electrical stimulation) failed at the orad end before it was lost at the caudad end. There were, however, many exceptions to these rules. The conclusion reached was that the second rise in the curve could not be eliminated by cutting away the bowel either immediately above or below the stimulated place.

Nicotin. The next experiment was to see what effect nicotin would have on the second rise. If, as is commonly taught (Bayliss and Starling, 1899, p. 136; Langley, p. 853) nicotin blocks synapses, it might change the appearance of the curve of contraction. Actually it did not do so even when lethal doses were used. We then repeated the experiments of Thomas and Kuntz, and Mulinos, and found as they did, that nicotin, even in enormous doses, does not always block the effects of vagal stimulation on the bowel. It may do so at first with small doses, but we have records showing good responses of the bowel to vagal stimulation in animals that had just died from an overdose of nicotin. In one case these responses continued for seven minutes after the apparent death of the animal.

These observations leave us with a choice between two alternatives: either to believe that there are no synapses between the vagus fibers and those of Auerbach's plexus or else that nicotin does not entirely block them. Because we have obtained evidence from experiments with asphyxiation that there is a synapse between the fibers in the vagus nerves and in Auerbach's plexus, the most probable explanation is that nicotin does not always produce a complete block. Nicotin certainly has some adverse influence on the nervous system of the bowel because in rabbits poisoned with this drug, peristaltic rushes rarely appear spontaneously, and when they are started artificially they travel in a peculiar way, which will be described in another article.

Nolf has described changes in conduction in the nicotinized bowel of the chicken which indicate that the drug has blocked synapses between long neurones in Auerbach's plexus but as yet we have been unable to obtain evidence of this in the rabbit. In our experiments nicotin did interfere with the conduction along the bowel of electrically produced disturbances, but the records suggested that this was due largely to a poisoning of the muscle and a weakening of the response to stimulation. Incidentally, M. Lapicque has shown that nicotin greatly lowers the irritability of muscle.

In our experiments with nicotin, when recording levers were fastened at intervals of 2 cm. along the bowel, it was found that the amplitude of contraction at the point stimulated and also at a distance failed about as fast as conduction failed. We never saw anything to suggest that conduction

failed abruptly at the end of a neurone. This does not necessarily militate against Nolf's views because it may be that in the rabbit an electrically produced disturbance, unless it gives rise to a peristaltic rush, cannot pass from one neurone to another. If this be true, and if we accept as a working hypothesis Nolf's diagrammatic plan of the myenteric plexus, our experiments must indicate that the long neurones which he pictures as forming a chain, are about 20 cm. in length.

Unfortunately, the problem is much complicated by the fact that when the excised intestine is stimulated at either end by a quick distention of a short loop with water, it is easy to obtain records showing signs of rapid conduction of the disturbance over long distances. The fact that this type of conduction persists for hours after the stoppage of the circulation, makes it appear very unlikely that there is any synaptic interruption in the nerve-paths involved.

Possible nature of the conducting paths. Figure 4, a and f, shows that conduction of an electrically produced disturbance normally takes place with a decrement. It is conceivable that this decrement is due to the progressive thinning of a bundle of neurones which overlap like shingles on a roof. Possibly against this conception is the fact that conduction takes place with a decrement in the first 15 cm. of the duodenum where one might expect the neurones to start out with about the same lengths. For the moment we are sure of only one thing and this is that we will always be reluctant to draw conclusions from any experiments done with nicotin.

Effect of degenerative section of vagus and splanchnic nerves. A surer method of getting rid of some of the postulated synapses in the bowel might be to cut the vagus and splanchnic nerves and allow their fibers to degenerate. This we did and found that the bowel continued to respond as before to the two types of current. Apparently, then, if the second rise is dependent on the presence of a synapse it must be situated in the mesenteric plexus distal to the vagal and splanchnic nerve-endings. Incidentally, degenerative section of the vagus nerves did not interfere with the conduction of electrically-produced disturbances, but a similar section of the splanchnic nerves did somewhat interfere.

Effect of asphyxia. Again with the hope of stopping conduction through synapses we deprived the bowel of its blood supply. It has been shown repeatedly that the most vulnerable part of the nervous system is the synapse. Those in the brain and spinal cord are so sensitive to anoxemia that their ability to conduct is irreparably lost within ten or fifteen minutes after the stoppage of the circulation. It is suggestive, therefore, to find as we did, that in the rabbit the effect on the bowel of faradic stimulation of the vagus nerves disappeared in from ten to twenty-seven minutes after the stoppage of the circulation. The inference is that most anatomists and physiologists are correct, and that there is a synapse between the fibers of the vagus nerves and those of Auerbach's plexus.

The presence of such a synapse would help to explain why stimulation of the vagus nerves so often fails to influence the bowel; it would explain the long latent period, it might to some extent explain the fact that the response is usually a mixture of stimulation and inhibition, and it would throw light on the fact that stimulation of one of the vagus nerves will often produce changes in the activity of the bowel at one point but not at other points nearby. Nolf's work indicates the presence of such synapses, and they have to be postulated, if only because there are not enough nerve fibers in the two vagus bundles to supply even a small fraction of the muscle fibers in stomach and bowel.

The presence of synapses in Auerbach's plexus was indicated by the fact that in a number of our animals the stoppage of the circulation in segments of bowel resulted, after two or three minutes, in a cessation of normal peristaltic rushes. If the loop was not too long the rushes would continue to pass, but only in a peculiar, incoördinated way, which will be described in another paper.

In seventeen years of experiment one of us (Alvarez) was never able to elicit a rush in a recently dead rabbit; there was usually one agonal wave and then no more. As yet we have no explanation for this sudden cessation of activity in the dead animal. If it were due purely to the effect of anoxemia on the nerves one would expect the rushes to continue for a few minutes as they do when, in the living animal, the circulation is cut off from part of the bowel. Recently we saw an imperfectly coördinated rush in a rabbit that had ceased to breathe ten minutes before. The salt solution in the tank was oxygenated. We are now trying to learn something about the conditions which made this rush possible.

If (with the tissues kept at a temperature of about 38°C.) synapses lose their function in from ten to twenty-seven minutes, the next question is: When, after the removal of segments of bowel from the body, will the nerve fibers lose their ability to conduct? One would expect, of course, that the survival time would be influenced by the temperature of the Locke's solution and by the amount of oxygen dissolved in it. Actually, in five out of eight experiments performed with bowel quickly removed from the animal and placed in unoxygenated but not boiled Locke's solution at 38°C., failure of conduction came after a remarkably constant interval. The time elapsed in successive experiments was 103, 105, 100, 110, and 110 minutes. In another experiment the interval was 123 minutes, but it was found that the thermostat had failed to function and the temperature of the bath had dropped to 33.5°C. In another animal conduction seemed to fail at 50 minutes and to return at 140 minutes, but the records were hard to interpret. In still another animal no sign of conduction could be detected after 40 minutes, although the irritability of the muscle was still good after six and a half hours.

One difficulty in making these experiments is that sometimes for a few minutes, the bowel will fail to react, possibly because of accidental changes in the resistance in the stimulating circuit, and the experimenter will be misled into thinking that the faculties of irritability and conduction have been lost. In some experiments we have greatly improved conduction, and have prolonged the time during which it can be obtained, by filling the loop of bowel with oxygen.

In another series of experiments the bowel was quickly removed from the animal and placed in Locke's solution at a temperature of 12°C. At intervals of twenty-four hours segments were transferred to warm oxygenated Locke's solution, and the power to conduct was always found to be lost at the first testing. The ability to respond to an electric stimulus and to contract rhythmically remained for several days. Although in some of the segments in which the power of conduction was lost there was a change to a galvanic type of response to the tetanizing current, this change was not constant and it did not always take place at the time when the nerves seemed to be losing their function. In many cases the bowel continued to respond with a composite type of curve long after the ability to conduct had been lost. It would seem from this that the second rise can hardly be ascribed to a stimulus arriving by way of the nervous system, but other observations which we are now making show that parts of the conducting mechanism of the bowel are remarkably resistant to anoxemia, and that caution must be exercised in interpreting the results of experiments.

One of the greatest difficulties encountered in this work arises in the fact that there appear to be at least two types of peristaltic rush; one in which the contractions in successive portions of the bowel are well coördinated by a synaptic nervous system, and another in which they are fairly well coördinated simply by the passage of a column of fluid. Strange to say, thus far we find it easier to differentiate these types with the unaided eye than with graphic records.

COMMENT. We regret that after all the work done, and much more that will be reported later, we are still in doubt as to the causes of the differences in the response of the bowel to different types of currents. There is much evidence to indicate that the steep galvanic type of contraction curve can be produced by a faradic current whenever the bowel is sensitive enough to respond with an initial sharp rise. This of course does not explain why the galvanic current commonly produces this sharp rise even in an insensitive bowel. Furthermore, a faradic type of response can be converted into what, at first glance, looks like a galvanic response whenever the first contraction of the muscle is too weak to lift the writing point of the lever. The distinguishing feature of this false galvanic response is a long latent period.

The great amplitude and the longer duration of contraction with inter-

rupted currents is almost certainly due to summation, and it is possible that, at least at times, the second rise can be explained in this way; the difficulty is that we cannot easily explain in this manner the second rise which sometimes appears in records made at a point distant from the stimulating electrodes (fig. 2, d, and 4, c.). In most cases the distant contraction is either of the galvanic type as in figure 4, b, or else it is indeterminate; that is, there is only an increase in the amplitude of a rhythmic contraction, as in figure 4, a and 4, f. In the second record (from the top) in figure 4, a and 4, f, there is no sign of summation in the transmitted disturbance, and the duration of contraction is short. Whenever there is summation, relaxation is usually slow. This being true, it is not easy to explain the composite curves in figures 2, d and 4, e as being due to a summation of stimuli. Figure 4, e shows that the composite curve is not necessarily transmitted as such because in this instance the response at the point of stimulation was of the simple galvanic type.

There is still the possibility that there are two types of contractile substances with different latent periods. It is hard otherwise to account for such a curve of contraction as is depicted in figure 1, t. It is possible also that more information might be secured by analyzing with the oscillograph the forms of the currents used as stimuli and then correlating these forms with the types of contractions obtained.

A single galvanic stimulus apparently does not affect either the extrinsic or intrinsic nerves of the bowel. It still is not clear why a rapidly interrupted galvanic current does not seem to affect these nerves when an even more rapidly interrupted faradic current does. It does not seem to be due to too short a chronaxie because the bowel will respond locally to condenser discharges of very short duration.

Our observations indicate that conduction along the bowel is dependent on neurones in the myenteric plexus. The fact that the rhythmic activity of the bowel persists for days after the power of conduction has been lost makes it appear highly improbable that nerve cells have anything to do with these spontaneous contractions.

Nicotin does not appear to be an entirely trustworthy interrupter of synapses. It acts peculiarly at times, and at present we are more inclined to trust to asphyxia as a means of blocking nervous impulses. Unfortunately the effects of asphyxia also vary, not only with the metabolic rate of the animal being studied, but with the temperature of the surrounding medium, and with the amount of hemoglobin left in the blood vessels.

Perhaps the most important result of this work is the discovery of ways in which the intestinal muscle can be stimulated without at the same time affecting the myenteric nerves. If we are correct in our observations and our assumptions we have supplied future workers in this field with certain tools which may some day be of value.

SUMMARY

In the small intestine of the rabbit, stimulation with a constant galvanic current produced a short simple curve of contraction with a sharp initial rise and a low amplitude. This disturbance never spreads far along the bowel. A tetanizing faradic current produced a curve of contraction with a slow initial rise and then, after about 1.4 seconds, a sharp rise; the amplitude was greater than that seen with the single galvanic stimulus, and usually there were signs of conduction of the resulting disturbances orad and caudad along the bowel.

With particularly irritable segments of bowel the tetanizing current often produces a sharp initial rise, and therefore a galvanic type of curve. In successive segments of excised bowel there was a distinct gradient in the percentage of such galvanic reactions obtained; it ranged from 60 per cent in the duodenum to 19 per cent in the lower part of the ileum.

A slowly interrupted galvanic current produced a curve with a large amplitude and usually a steep initial rise; conduction was good. With more rapid interruption of the galvanic current the local response of the bowel was poor and no conduction was seen. Single strong induction shocks commonly failed to produce any effect on the bowel. Strong, quick condenser discharges produced a local contraction of galvanic type.

The fact that the extrinsic nerves of the bowel did not respond to single shocks or to a constant current, the fact that these stimuli did not give rise to a traveling disturbance along the bowel, and observations showing that conduction along the bowel is due to nerves, all indicate that the intrinsic nerves of the bowel are not influenced by a single make-or-break stimulus or by the passage of a constant current.

The second rise in the faradic curve did not appear to be due to a difference in rate of contraction of the longitudinal and circular coats of muscle. There is evidence to show that it is due to a stimulus arriving by way of the nervous system, but other observations make this appear improbable. There is much evidence to indicate that it is produced by a summation of stimuli, but there are reasons also for doubting this.

The latent period between stimulation of the vagus nerves and a response in the bowel varied from 0.4 to 3.2 seconds with a mode of about 1.1 seconds. The response was usually a mixture of inhibition and stimulation; it was often absent, and it was always fleeting. It was demonstrable at certain points along the bowel but not at others nearby.

Nicotin interfered with conduction of an electrically produced disturbance along the bowel but this seemed to be due as much to injury of muscle as to injury of nerve. Contrary to the generally accepted view, nicotin does not seem to be an entirely trustworthy blocker of synapses. It interfered with the passage of peristaltic rushes through loops soaked in weak solutions of the drug, but rarely stopped them.

Stoppage of the circulation led to the abolition, within from ten to thirty minutes, of the response of the bowel to stimulation of the vagus nerves. This was thought to indicate the presence of a synapse between vagal and myenteric neurones. This synapse was usually blocked by small doses of nicotin, but not by large or even lethal ones.

Stoppage of the circulation for about ten minutes caused the loss of the finer coördination of peristaltic rushes. The same change was noted in the nicotinized bowel, and was interpreted as due to the failure of function in synapses. Conduction along the bowel of disturbances produced by faradic stimulation failed after an interval of approximately 105 minutes after stoppage of the circulation. This was thought to indicate a cessation of function in nerve fibers.

The difference in the shape of the curves of contraction obtained with constant and interrupted currents usually remained unchanged after either nicotinization or anoxemia had destroyed the ability of the intestinal nerves to transmit an electrically produced disturbance.

The fact that the rhythmic contractions of the bowel usually persisted for several hours after the loss of its power to conduct indicated that these contractions are not dependent on nerve cells. The irritability of the muscle generally persisted for some time after the rhythmic contractions had ceased.

BIBLIOGRAPHY

ALVAREZ, W. C. 1924. This Journal, lxix, 229.
ALVAREZ, W. C. AND K. HOSOI. 1929. This Journal, lxxxix, 182.
1930. This Journal, xciv, 448.
BAYLISS, W. M. AND E. H. STARLING. 1899. Journ. Physiol., xxiv, 99.
FLEISCH, A. AND W. H. WYSS. 1923. Pflüger's Arch., cc, 290.
KLEE, P. 1921. Pflüger's Arch., cxlv, 557.
LANGLEY, J. N. 1903. Ergebn. d. Physiol., ii², 818.
LAPICQUE, L. AND I. MEYERSON. 1912. Compt.-rend. Soc. de biol., lxxii, 63.
LAPICQUE, M. (quoted by H. FREDERICQ). 1928. Physiol. Rev., viii, 501.
1921. Compt.-rend. Soc. de biol., lxxxiv, 654.
MEEK, W. J. 1910. This Journal, xxv, 367.
MELIK-MEGRABOW, A. M. 1926. Pflüger's Arch., ccxiii, 245.
MILLER, F. R. AND R. A. WAUD. 1925. This Journal, lxxiii, 329.
MULINOS, M. G. 1927. Proc. Soc. Exper. Biol. and Med., xxv, 49.
NOLF, P. 1928. Arch. internat. de physiol., xxx, 317.
ROGERS, F. T. AND Z. BERCOVITZ. 1921. This Journal, lvi, 257.
THOMAS, J. E. AND A. KUNTZ. 1926. This Journal, lxxvi, 598.
VEACH, H. O. 1925. This Journal, lxxi, 229.

SOME EFFECTS OF FRESH PITUITARY HOMO-IMPLANTS AND OF THE GONAD-STIMULATING SUBSTANCE FROM HUMAN PREGNANCY URINE ON THE REPRODUCTIVE TRACT OF THE MALE RAT[1]

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The pioneer work of Smith (1), Smith and Engle (2) and Zondek and Aschheim (3) has demonstrated clearly that anterior lobe of the hypophysis, as well as the urine of pregnant women, causes marked stimulation of the reproductive system. The response of the female reproductive tract to such stimulation has received more attention than has that of the male. Smith and Engle noted a marked response of the male accessory reproductive organs to fresh homo-implants in rats and mice. The absolute testicular weight in young rats treated with homo-implants for ten days or longer was always greater than that of the control, though in many cases the difference was slight. Subsequently, many reports have continued to emphasize the response of the accessory organs of the male to fresh gland implants, injections of human pregnancy urine or extracts from pregnancy urine.

In connection with a study of the effects of gonad hormone administration to rats (4) it became desirable to employ fresh homo-implants in combination with different hormone injections. Hence it was necessary to become acquainted with the effects of fresh tissue implants upon the testis and accessory organs in order to interpret observations following the introduction of hypophysis tissue in combination with sex hormones. We have been able also to study the effects on the male of hebin, the gonad-stimulating substance prepared from human pregnancy urine by Wallen-Lawrence and Van Dyke (5), and to compare the histological effects produced with those obtained from fresh tissue implants.

We take great pleasure in making acknowledgments to Wallen-Lawrence and Van Dyke for the gonad-stimulating substance called by them "hebin." The prepararation employed by us had been determined by them to cause precocious sexual maturity when daily doses of approximately 0.06 cc. were

[1] This investigation has been aided by a grant from the Committee on Sex Research of the National Research Council; grant administered by Prof. F. R. Lillie.

injected into rats from the twenty-first day of age until they were sacrificed on the twenty-sixth day.

Hypophysial homo-implants in young males. The effects of both fresh implants and of hebin have been determined from an examination of the testes (gross size, spermatogenetic maturity, seminiferous tubules diameter, and interstitial cell conditions), the prostate gland (relative gross size and microscopic differentiation), and the seminal vesicles (gross size, secretion contents and cytological differentiation). The effects of gonadal stimulating substances in combination with sex hormone administrations are reported elsewhere (5).

The results obtained from fresh tissue implants into young normal males are indicated by the summary of observations upon four males listed in litters 1, 2 and 3 of table 1; ten young animals gave results consistent with these.

The following explanation for litter 1, table 1, will enable one to interpret this summary of observations for the animals receiving fresh implants as well as those injected with hebin. The litter of five males consisted of two treated with fresh implants, and three controls. The two former were given two daily homo-implants of whole hypophysis from the twenty-fifth to the thirty-second day and were killed on the thirty-third day after birth.

The testis measurements of the untreated thirty-three-day control (273A) were 13 x 7.5 mm. Spermatozoa had not yet appeared in the seminiferous tubules. The average diameter of twenty-five tubules, measured with the aid of an ocular micrometer, was 0.14 mm., and the interstitial cell mass was rated unity to afford a rough means of comparison with the treated testes. The prostate gland had attained the characteristic secretory differentiation, for this has usually appeared by the fifteenth day. The gross size of seminal vesicles was 6 x 2 mm., and the epithelium had not yet differentiated secretion granules, which usually appear in the normal vesicle about the thirty-fifth day.

When one compares the results obtained from administration of sixteen fresh homo-implants (animal 271C) with the untreated control, it becomes evident that testis size and tubule diameter are suggestively greater; the number of cells in the intertubular spaces is approximately 50 per cent greater; seminal vesicle size is markedly increased, and secretory differentiation approaches the normal adult state. The measurements of seminal vesicles are much inferior to weight records because the shape and nature of the vesicle make it impossible to adequately express the great volume increase noted. However, since we rely upon cytological differentiation to express effects, rapid fixation was necessary and weights were not determined. A few spermatozoa were to be seen in the seminiferous tubules of the treated males, and they were not present in those from a control littermate. Littermate controls two and five days older possessed spermatozoa.

TABLE 1

Showing the effects upon young male rats of fresh hypophysial implants (litters 1, 2 and 3) and of urinary hebin injections (litters 4 to 9)

Spermatozoa when seen are indicated as + and when absent as −. Seminiferous tubule diameter is the average diameter determined for twenty-five tubules by ocular micrometer measurements. Interstitial cell mass in each litter is rated as unity for the control untreated animal (amounts estimated by cell counts in interstitial spaces throughout the testis). Prostate gland in all cases is rated to show attainment of secretory state. Secretion granules in seminal vesicles are rated from 4 + (the normal adult condition) to − when no secretion granules are present.

LITTER	ANIMAL	TREATMENT	AGE AT DEATH	TESTIS SIZE	SPERMATOZOA IN SEMINIFEROUS TUBULES	SEMINIFEROUS TUBULE DIAMETER	INTERSTITIAL CELLS	PROSTATE GLAND	SEMINAL VESICLES	
									Size	Secretion granules
			days	*mm.*		*mm.*			*mm.*	
1	271C	16 rat hypophysis implants (25–32 days)	33	14 x 8	+*	0.17	1.5	+	11 x 3	+++
1	272C	16 rat hypophysis implants (25–32 days)	33	14 x 8.5	+*	0.15	1.5	+	9 x 4	++
1	273A	Littermate control	33	13 x 7.5	−	0.14	1	+	6 x 2	−
1	274A	Littermate control	35	13 x 7	+*	0.16	1	+	7 x 2.5	+
1	275A	Littermate control	38	15 x 9	+	0.17	1	+	9 x 3	+++
2	269C	18 rat hypophysis implants (25–35 days)	36	15 x 9	+	0.17	1.5	+	11 x 5	++++
2	270A	Littermate control	36	14 x 9	+	0.17	1	+	6 x 3	+++
3	182C	20 rat hypophysis implants (20–39 days)	40	15 x 8	+	0.16	4	+	12 x 7	++++
3	181A	Littermate control	40	15 x 9	+	0.17	1	+	7 x 2.5	+++
4	202C	Hebin 11–27 days	28	11 x 7	−	0.12	15	+	11 x 5	++++
4	203C	Littermate control	28	10 x 6.5	−	0.12	1	+	5 x 1.7	−
5	262C	Hebin 12–23 days	24	9 x 5.5	−	0.11	4	+	8 x 5	+++
5	265C	Hebin 12–27 days	28	10 x 5.5	−	0.12	1.5	+	7 x 3.5	−
5	266A	Littermate control	28	9 x 5.5	−	0.12	1	+	4 x 2	−
5	268C	Hebin 12–30 days	31	11 x 7	−	0.12	4	+	10 x 5.5	++++
6	251C	Hebin 21–24 days	25	11 x 6.5	−	0.11	4	+	6 x 3	+
6	256C	Hebin 21–28 days	29	11.5 x 6.5	−	0.12	7	+	7 x 3	++
6	257A	Littermate control	29	11 x 6	−	0.12	1	+	6 x 2	−

* Only three or four sperm heads seen.

TABLE 1—*Concluded*

LITTER	ANIMAL	TREATMENT	AGE AT DEATH	TESTIS SIZE	SPERMATOZOA IN SEMINIFEROUS TUBULES	SEMINIFEROUS TUBULE DIAMETER	INTERSTITIAL CELLS	PROSTATE GLAND	SEMINAL VESICLES	
									Size	Secretion granules
			days	*mm.*		*mm.*			*mm.*	
7	250C	Hebin 22–25 days	26	10.5 x 6	−	0.12	2	+	5.5 x 2	+
	254C	Hebin 22–29 days	30	13 x 7.5	−	0.13	3	+	8.5 x 4	++++
	255A	Littermate control	30	10 x 5.5	−	0.11	1	+	5 x 1.5	−
8	252C	Hebin 30–34 days	35	15 x 8.5	−	0.15	4	+	10 x 4	++++
	258C	Hebin 30–39 days	40	11.5 x 7	−	0.13	8	+	8 x 3	++++
	259A	Littermate control	40	9 x 6.5	−	0.11	1	+	5 x 1.5	−
	267C	Hebin 30–49 days	50	16 x 9.5	+	0.17	6	+	14 x 6.5	++++
9	253C	Hebin 30–36 days	37	10 x 6.5	−	0.13	2	+	6 x 2.5	++
	260C	Hebin 30–40 days	41	13 x 8	+*	0.14	3	+	7 x 3	++++
	261A	Littermate control	41	11 x 6.5	−	0.13	1	+	5.5 x 2	−
	263C	Hebin 30–46 days	47	13 x 9	+	0.17	1.5	+	12 x 5.5	++++
	264A	Littermate control	47	14 x 9.5	+	0.18	1	+	7.5 x 4	++++

In general, fresh homo-implants given young male rats has caused a material hastening of secretory differentiation of the seminal vesicles and has caused a great increase in their gross size. It has led to marked increases in size of the prostate gland, but after the attainment of secretory differentiation in this gland, a histological study is inadequate to suggest grades of secretory intensity. The fresh implants have led to material increases in the amount of cells in the intertubular spaces of the testis; in litter 3 the increase was approximately 300 per cent. Neither testicular gross size nor average seminiferous tubule diameter has been modified consistently by treatment. The earlier spermatozoön appearance in the treated males of litter 1 (two days earlier) has not always been obtained, for other cases sacrified just preceding and just after spermatozoön appearance failed to show an earlier appearance, or a greater number, of these cells in the treated animals. We do not consider the suggestive earlier appearance in the case of litter 1 as significant.

Injection of urinary hebin into immature males. Litters 4 to 9 (table 1) have been selected to show the effects of subcutaneous injections of hebin (Wallen-Lawrence and Van Dyke), the urinary gonadal stimulating substance.

The general effects from hebin have varied only in degree from those noted from fresh implants. Both urinary hebin and fresh homo-implants

gave results attributable to a stimulation of the testicle, since neither was effective in the castrated animal. The stimulating effect appeared to involve only the interstitial tissue, and this has been correlated with an earlier, and a greater secretion of testis hormone as indicated by the microscopical study of the accessory reproductive organs (for details of hormone indicators see 6, 7 and 8).

The data recorded in table 1 fail to show consistent differences between treated and untreated males in respect to gross testicle size, time of spermatozoön appearance, seminiferous tubule diameter, or state of spermatogenetic differentiation. There can be seen a fairly consistent difference in interstitial tissue, for in all but one or two pertinent exceptions, discussed below, that in the treated animal exceeds the amount in the control testis; there is likewise a consistent increase in size of the prostate and seminal vesicles of treated males. The increased secretory stimulation of the seminal vesicles noted from microscopic preparations is of greater importance than size relations.

Particular attention is directed to litters 4 and 5. Animal 202C was killed at the age of twenty-eight days after seventeen daily injections of $\frac{1}{8}$ cc. of urinary hebin. The volume of its seminal vesicles and prostate was estimated to be roughly five to seven times the volume of these organs in the littermate control; actual measurements showed a pronounced increase in the size of the vesicles. From a microscopical study, the vesicles of the control were found to be in an infantile state, whereas the treated male possessed seminal vesicles cytologically indistinguishable from those of adult normal males. The testes of these two animals showed differences only in amounts of interstitial tissue; that of animal 202C is rated as fifteen times the amount in the control testis. Figures 1 and 2 show photomicrographs of the testis and seminal vesicles of the treated male, and figures 3 and 4, the testis and seminal vesicle of the untreated littermate control. The parallelism of extreme interstitial cell development, without other testis differences, and the extreme development of the accessory organs of reproduction is very suggestive that hormone is being secreted in the testis by interstitial cells.

Animal 265C (litter 5) is important for the fact of its weak response to hebin injection and because it emphasizes the relationship existing between precocious response and interstitial cell hypertrophy. Seminal vesicles of this animal, killed at twenty-eight days of age, were slightly larger than those of the untreated littermate (266A), but had not yet developed to a secretory state. A treated littermate receiving the same injections as 265C but killed at twenty-four days after birth (262C) had larger seminal vesicles showing a definite cytological response to the injections; the secretory state compared favorably with the normal adult state. The interstitial cell response was marked in the treated male killed at twenty-four days of

age (rating 4) whereas in that killed at twenty-eight days, with four additional injections, only a relatively slight response was perceptible (rating 1.5). In this case, therefore, a lack of interstitial cell response is paralleled

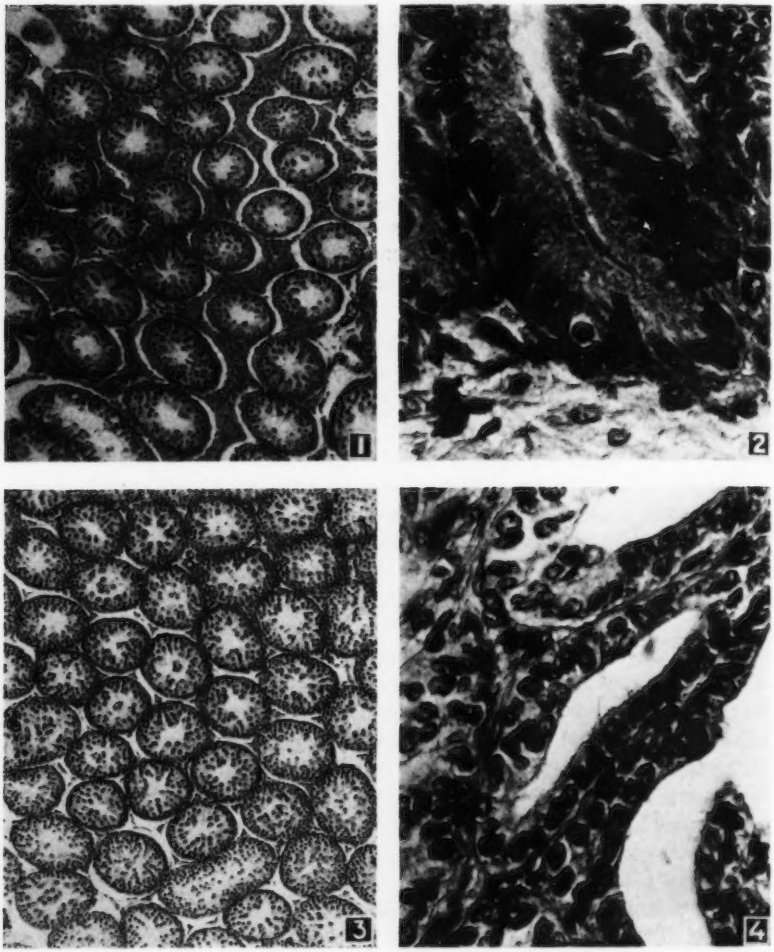

Fig. 1. Portion of testis from animal 202C at 28 days of age (hebin injections from 11 to 27 days). ×100.

Fig. 2. Seminal vesicle from same animal as figure 1. ×1000.

Fig. 3. Testis of 28 day control untreated male 203C. ×100.

Fig. 4. Seminal vesicle from same animal as figure 3. ×1000.

by a lack of seminal vesicle development. We have no suggestion to make as to the cause of the lack of response to hebin injections in this case, but we would like to emphasize the interstitial cell-seminal vesicle relationship that is evident.

The cytological differentiation of the seminal vesicles has proved to be a far more dependable indicator for the testis hormone than has the gross size of these organs. However, the product of seminal vesicle length times width has been suggested by some writers as a hormone index for the mouse (9). Animals 270A and 265C offer an interesting comparison, for seminal vesicles of the latter present the larger dimensions yet show weaker secretory activity. Other cases in the table illustrate that comparative gross size and secretory differentiation do not always run parallel. Our experiences with adults, particularly where castration was performed post-pubertally, have emphasized many times that with the exception of maximal reactions the gross size of the seminal vesicle is entirely undependable as a hormone index; cytological study, on the other hand, makes possible very definite indices of hormonal states of the animal.

The administration of the gonad-stimulating substances (hebin, as well as fresh homo-implants) into immature males has elicited a structural response of the testicle only by an increase of interstitial tissue. Gross size, tubular size, or spermatogenetic differentiation have not been appreciably modified. Increase in gross size and marked secretory stimulation of the accessory organs, more evident after injections of urinary hebin, clearly demonstrate that the testicle has responded physiologically by excessive secretion of testis hormone.

Effect in normal and castrated adult males. Injections of the urinary gonad-stimulating substance into adult normal males have given results quite similar to those observed in immature males; injections into castrated males have been ineffective. The observations can be briefly considered by citation of details on a typical case of each type.

Animal 245A, an adult male of 175 grams weight and in good reproductive condition, was opened under ether anesthesia for the removal of one testis and one seminal vesicle; these were preserved for microscopical study. Such a procedure enables one to learn the condition of the testicle in two respects—i.e., its gametogenetic state shown by histological study of the organ, and its endocrine state as revealed by the microscopic character of the seminal vesicle. Caliper measurements of the seminal vesicle were 17 x 8 mm. One day after operation and for seventeen days thereafter the animal received subcutaneous injections of three-fourths of a cubic centimeter of hebin, and was killed one day after the last injection. The measurements of the remaining seminal vesicle at this time were 23.5 x 11 mm. The prostate gland was recorded as "huge" in comparison with a control, unilateral castrate which had been caged with animal 245A but had not received injections.

The testicles removed before and after the seventeen daily injections of hebin were similar in all details. Both were normal. The interstitial cell material appeared slightly more pronounced in the testicle removed after injection, but our methods employed were inadequate to accurately express small differences. The increase was not marked.

A comparison of the seminal-vesicle measurement before and after injection, and our estimate of seminal vesicle volume compared with that of an uninjected, similarly operated male, reveals a great increase in the size of this structure following the injection period. The second vesicle is in a state of much greater activity than the one removed before injections were begun. The photomicrograph figure 5 shows the histological character of the vesicle removed before injection, and figure 6, the second vesicle removed at the end of the injection period. Here the secretion granules are much more numerous and conspicuous.

The prostate glands of treated animals have been uniformly larger than those of untreated animals with otherwise similar histories. The position and shape of the prostate prevents accurate measurements, and its cytological characteristics are inadequate to express degrees of activity in excess of an active normal state. In general, the prostate glands of treated animals were more distended with secretion than were those from uninjected animals.

Injections of hebin into ten adult males, with one or both testes present, have been without perceptible effect upon the testes, with the exception of strong indications of interstitial-cell increase. The effects upon the accessory organs of reproduction were marked by an increase in size, and more intense secretory activity, on the part of the seminal vesicles. It follows, therefore, that more hormone was produced during the period of injection in the animal with one testicle present, than had been produced by both testes before injection.

The observations on castrated males receiving similar hebin injections to the above can be illustrated by referring to one animal, since six animals gave consistent findings.

Animal 243A, an adult male of 180 grams, was operated for removal of both testes and one seminal vesicle; measurements of the latter were 18 x 9 mm. One day after operation and for seventeen days this castrated male received injections of three-fourths of a cubic centimeter of urinary hebin, and it was sacrified on the day after the last injection. The remaining seminal vesicle measurements were 11 x 5 mm.

Microscopical study of the testicle and seminal vesical removed before injection showed them to be normal, and figure 7 illustrates the state of the secretory epithelium of the latter. The second seminal vesicle, removed at the end of the injection period, was much smaller and more shrunken than just after castration. Its histological character is seen from figure 8 where the castrate type of the vesicle is shown.

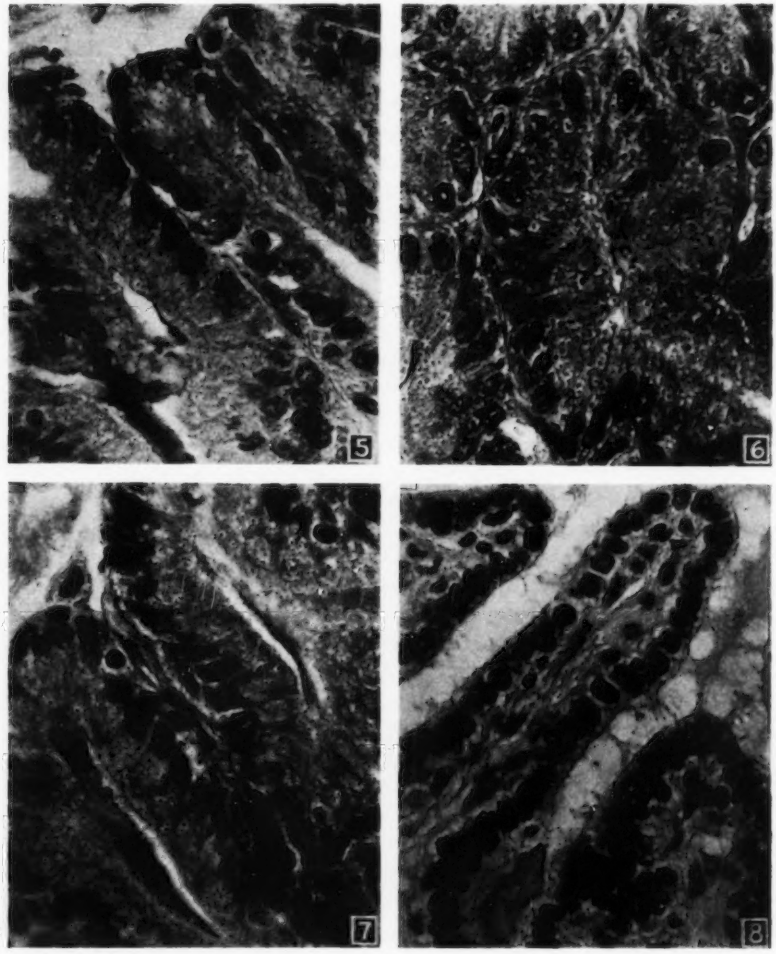

Fig. 5. Seminal vesicle from animal 245A removed before injecting hebin. ×1000.

Fig. 6. Opposite seminal vesicle from same animal as figure 5 after 17 daily injections of hebin. ×1000. Note greater activity of secretory epithelium.

Fig. 7. Seminal vesicle from normal male 243A at time of castration. ×1000.

Fig. 8. Opposite seminal vesicle from same animal as figure 7 after 17 injections of hebin (testes removed). The typical castrate condition shows hebin ineffective when testes are absent. ×1000.

The detailed observations described are typical for all cases of these two types of treatment on adult males. They show that the gonad-stimulating substances are ineffective when testes are absent, but powerful stimulants when testes are present.

Discussion. Our study of the responses of the male reproductive system to fresh hypophysial homo-implants as well as to injections of the substance obtained from pregnancy-urine confirms and extends the effects noted by others.

Smith and Engle (2) could not detect an effect on the reproductive system of normal adult male rats from fifteen daily fresh homo-implants but ten implants in young males resulted in some increase in testis size; acceleration of spermatogenesis was not observed. The accessory organs gave a marked response. Steinach and Kun (12) obtained marked stimulation of the rat accessory reproductive organs from aqueous extracts of fresh beef hypophysis. Voss and Loewe (10) after one implantation of fresh sheep hypophysis into mice noted marked accessory gland stimulation, and report also a hastening of spermatozoön appearance in treated males. The small number of animals treated and our own observations with fresh implants suggest the advisability of confirmative findings before acceptance of spermatogenetic stimulation.

In our experience the only consistent effect on the young testis was an increased development of the interstitial tissue. Spermatogenesis was not hastened. The accessory organs were markedly increased in size and showed a marked precocious secretory differentiation. Corey (11) believed it possible to detect effects upon the rat testis as early as fifteen days after birth.

Injection of hebin from pregnancy urine has given effects different only in degree from those following fresh tissue implantation and this is undoubtedly to be attributed to greater concentration of the stimulating principle.

Untreated human pregnancy-urine injected into young males has been found to exert a marked stimulation upon the reproductive accessories (13, 14, 15, 16) but it has not led to precocious spermatogenesis; interstitial cell hypertrophy has usually been noted. Laurent (16) reports greater effects on interstitial cell hypertrophy from urine of men than from pregnancy urine, but this has not been confirmed to our knowledge.

Extracts from pregnancy-urine have likewise proven effective in stimulating interstitial cell hypertrophy and size increases in the accessory organs (17, 18, 19, 20, 21). Borst and Gastimirovic (19) alone report acceleration of spermatogenesis from "Prolan" injections but the effect is reputed to be on spermatogonial and spermatocyte divisions and does not lead to an earlier appearance of spermatozoa.

In our experience the effects produced on the male reproductive system

have been qualitatively alike in the administration of fresh rat hypophysial tissue or of the urinary substance hebin. In both treatments of immature male rats, interstitial tissue increase was prominent but spermatogenesis was not notably stimulated. The increase in size of the accessory reproductive organs involved also a real precocious secretory differentiation. The parallelism of effects from fresh implants and pregnancy-urine extracts is not considered by us as evidence that the urinary stimulating substance is of hypophysial origin. There are many facts pointing to the opposite conclusion.

SUMMARY AND CONCLUSIONS

1. Fresh hypophysial implants or injections of urinary hebin—the gonad-stimulating substance from human pregnancy urine (Wallen-Lawrence and Van Dyke)—have been followed in prepubertal male rats by:

a, slight increases in gross measurements of the testis; little or no increase in tubular diameter; little, if any, acceleration of spermatogenesis; and decided increase in the interstitial tissue.

b, decided increases in size of the prostate gland and seminal vesicles.

c, marked precocious attainment of secretory differentiation in the seminal vesicles.

2. Urinary hebin injections into normal adult males produced a marked size increase in prostate glands and seminal vesicles; secretion granules were both more numerous and larger. Visible effects on the testis were limited to slight increases in interstitial tissue.

3. Injections of urinary hebin into castrated males were without effect upon the reproductive tract.

BIBLIOGRAPHY

(1) Smith, P. E. 1925. Proc. Soc. Exp. Biol. and Med., xxiv, 131.
(2) Smith, P. E. and E. T. Engle. 1927. Amer. Journ. Anat., xl, 159.
(3) Zondek, B. and S. Aschheim. 1927. Arch. f. Gynak., cxxx, 1.
(4) Moore, C. R. and D. Price. 1930. Proc. Soc. Exp. Biol. and Med., xxviii, 38; 1932 (in press).
(5) Wallen-Lawrence, Z. W. and H. B. Van Dyke. 1931. Journ. Pharm. Exper. Therap., xliii, 93.
(6) Moore, C. R., D. Price and T. F. Gallagher. 1930. Amer. Journ. Anat., xlv, 71.
(7) Moore, C. R. and T. F. Gallagher. 1930. Amer. Journ. Anat., xlv, 39.
(8) Moore, C. R., W. Hughes and T. F. Gallagher. 1930. Amer. Journ. Anat., xlv, 109.
(9) Martins, T. 1930. Endokrinol., vii, 180.
(10) Voss, H. E. and S. Loewe. 1928. Pflüger's Arch., ccxvii, 604.
(11) Corey, E. L. 1928. Proc. Soc. Exp. Biol. and Med., xxv, 498.
(12) Steinach, E. and H. Kun. 1928. Med. Klinik., pp. 524–529.
(13) Fels, E. 1928. Zeitschr. f. Geburtshilfe, xciii, 50.
(14) Engle, E. T. 1929. Anat. Rec., xlii, 187.

(15) Bourg, R. 1930. C. R. Soc. Biol., civ, 1046.
(16) Laurent, G. 1930. C. R. Soc. Biol., civ, 115.
(17) Zondek, B. and S. Aschheim. 1928. Klin. Wochenschr., no. 18.
(18) Borst, M. 1930. Deutsch. Med. Wochenschr., lvi, 1117.
(19) Borst, M. and D. Gostimirovic. 1930. Münch. Med. Wochenschr., no. 36.
(20) Kraus, A. J. 1930. Proc. 2nd International Congress for Sex Research (London).
(21) de Jongh, S. E. and Dingemanse. 1930. Arch. Néerland de Physiol., xv, 470.

Photomicrographs prepared by Kenji Toda.

THE PASSAGE OF MATERIALS THROUGH THE INTESTINAL WALL

I. The Relation Between Intra-Intestinal Pressure and the Rate of Absorption of Water

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Starling (1920) suggested, on theoretical grounds, that the colloid osmotic pressure of the blood plasma or lymph of the villi may be responsible for the absorption of fluids by the intestine. In spite of the success which has resulted from the application of Starling's methods of study to the problems of fluid interchange in general, no one has, to my knowledge, made any attempt to subject this particularly promising suggestion to the test of experiment. As a matter of fact, however, one worker has made the test, but without reference to this particular point. Hamburger (1896) obtained evidence which led him to believe that absorption of fluid ceases when the intra-intestinal pressure falls to atmospheric or below. If this evidence were acceptable it would provide sufficient grounds for ruling out any appreciable effect of colloid osmotic pressure on absorption; for if the tissue fluids of the villi exert a colloid osmotic pressure, absorption of fluid from the gut must continue until the intra-intestinal pressure has been reduced to a negative value, numerically equal to the absorbing force of the osmotic pressure.

The data to be presented indicate that Hamburger was misled and that absorption of water from solutions of glucose and of isotonic sodium chloride continues down to negative pressures of considerable magnitude. Absorption ceases at a definite negative pressure, the value of which remains constant in any given animal over long periods of time. The same constancy exists for the absorption rate at any pressure above that which abolishes absorption. Consequently a definite absorption curve may be plotted for each animal, and such curves show that the rate of absorption is directly proportional to the amount by which the intra-intestinal pressure exceeds the pressure at which absorption ceases. The whole relationship between pressure and rate may be expressed most concisely in the form of a simple equation for a straight line:

$$y = k\,(x - b)$$

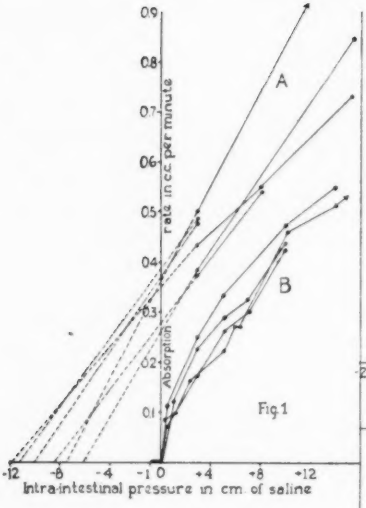


Fig. 1

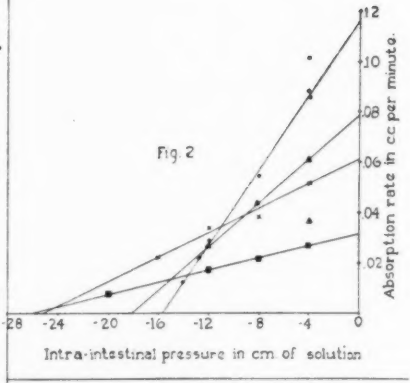
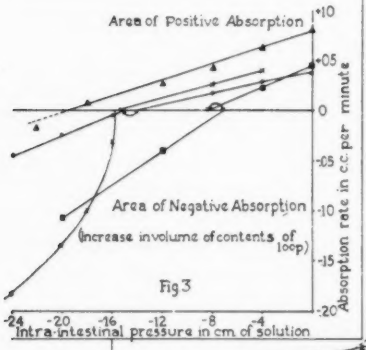


Fig. 2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Area of Negative Absorption
(Increase in volume of contents of loop)
Absorption rate in c.c. per minute
Intra-intestinal pressure in cm. of solution

Fig. 3

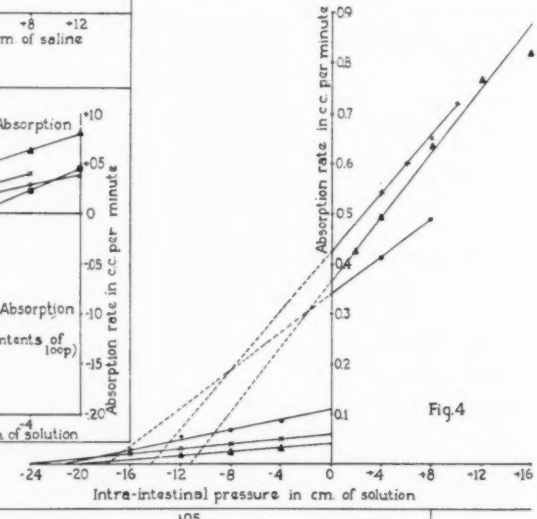

Fig. 4

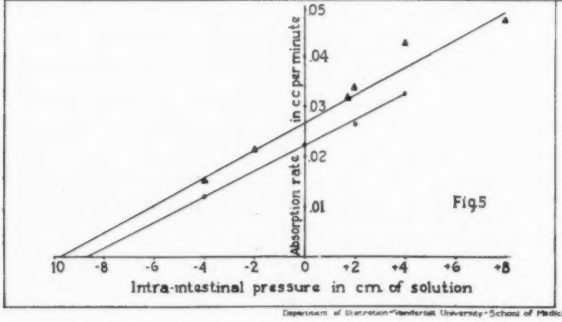

Fig. 5

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where y is the rate of absorption at any given pressure, k is the constant of proportionality, x is the intra-intestinal pressure, and b is the pressure at which absorption ceases.

Due to the fact that Hamburger's conclusions are so generally accepted as to be included in almost every discussion of the mechanism of intestinal absorption, it has seemed advisable to present an analysis of his data and methods, in order that the apparent discrepancy between his results and mine may be satisfactorily explained. This author found that the rate of absorption of isotonic saline solution from isolated loops of the dog's intestine was increased by raising the intra-intestinal pressure above atmospheric, but that absorption appeared to cease at atmospheric pressure. At negative pressures down to -8 cm. of solution there was no evidence of a change in volume of the fluid introduced. In the original article the data are merely tabulated. In figure 1 the average rates of absorption, which I have calculated from his data, are plotted against corresponding pressures. The points are arbitrarily connected by straight lines. In series A there is only one experiment from which it is possible to formulate any idea as to the actual form of the absorption curve. In this one experiment we seem to deal with something approaching a straight line. On the assumption that the true curves are straight lines, extrapolation to zero absorption rate carries the lines definitely into the region of negative pressure. The curves of series B, in which the pressure was actually lowered to -1 cm. in each case, show several features of difference from series A. In the first place, although the isolated loops used were of approximately the same length as those of the first series, the absorption rates are uniformly much lower. I believe that this may be attributed to interference with the circulation due

Fig. 1. (From data of Hamburger.) Relation between intra-intestinal pressure and rate of absorption of physiological saline. Isolated loops of small intestine of dog, each approximately 17 cm. in length. Points represent single determinations or averages.

Series A: gut loops supported on outside only. Series B: wall of gut supported by apparatus on inside and outside. Distinct evidence of interference with absorption.

Fig. 2. Relation between negative intra-intestinal pressure and rate of absorption, with wire coil supporting inside of loop. Squares represent results of experiments with 4 per cent glucose. The other data were obtained with 0.9 per cent salt solution.

Fig. 3. Curves of positive and negative absorption rates at negative intraintestinal pressure, with extrapolation in both directions to determine the pressure at zero rate. (Salt solution: squares and triangles. Glucose: crosses and dots.)

Fig. 4. Absorption rates for salt solution at positive and negative pressures, showing the failure of extrapolations of the corresponding curves to intersect at zero rate. Wire coil used to support the gut at negative pressures.

Fig. 5. Continuous absorption curves showing the straight line relation of rate to pressure which obtains when the absorbing surface is kept constant. Special intestinal clamp. Physiological saline solution.

to the presence inside of the loop of a basket-like wire support used to prevent collapse of the gut walls at atmospheric and negative pressures. This support consisted essentially of 6 wires which were situated *parallel* to the long axis of the gut. As the larger veins run *transversely* around the gut it would seem that the collapse of the intestinal wall against the wires would exert enough pressure across the veins to interfere seriously with the flow of blood. It is my experience that such interference must be carefully avoided in all absorption studies. The downward curving of the lines of series *B* can also, I believe, be explained on the same basis of interference with the circulation. Hamburger considered that the intra-intestinal pressure was atmospheric when the level of fluid in the open end of the connecting system was in the same horizontal plane with the lower surface of the isolated loop. Under these conditions the mucosal surface will actually be at a negative pressure, and the mechanical factors tending to block the circulation will be in operation over the whole gut surface. Not until the pressure has been raised to +2 cm., or thereabouts, will the whole surface be pushed away from the wire support. It is to be expected therefore that the points corresponding to pressures less than +2 cm. will tend to fall relatively lower than those above this point.

At atmospheric pressure absorption may have actually ceased, or at least have fallen to such a low rate as to have been impossible of certain detection by the method used. For the purpose of determining absorption rates, Hamburger employed a funnel with a scratch mark on the stem. As the level of fluid fell, additional fluid in known amounts was added occasionally, and the time for the level to fall to the mark recorded. Obviously such a scheme does not provide for the accurate determination of small volume changes.

The successful determination of absorption rates depends on the proper choice of methods and apparatus. For this reason, certain details of the procedures employed in my experiments will be presented before the actual data are considered.

Methods. In most of the experiments a helical coil of aluminum wire with loops separated by not more than 0.5 mm. was used to support the gut wall against the pressure of the atmosphere. With such a device the pressure lines are parallel to the large veins, the pressure is distributed evenly over a large fraction of the total surface and observations at negative pressure can be carried out for many hours without evidence of interference with the circulation. As would be expected the absorption rates are relatively slow when so large a part of the wall of the gut loop is in contact with the wire coil, for the absorbing surface is thereby reduced. The coil was made by winding no. 18 aluminum wire closely around a rod, 6 mm. in diameter, for a distance of 14 cm. The coil was then removed and pulled out uniformly to a length of 21 cm. Glass tubes, approximately 5

cm. long, were slipped in at each end for a distance of about 5 mm. Soft rubber tubing, $\frac{3}{16}$ inch bore by $\frac{1}{8}$ inch wall, was slipped over each glass tube and over the first few coils of the wire support. The ends of the rubber tubes were tapered smoothly so that on inserting the coil there would be no damage to the mucosa from sharp edges. A constriction in each glass tube beneath the rubber allows the ends of the gut loop to be tied tightly against the rubber without danger of the gut slipping off. The outside diameter of the finished coil is 8 mm. and that of the rubber sleeve at each end is 11 mm.

Dogs, ranging in weight from 6 to 14 kilograms, have been used as experimental animals. It was found that they had to be free from hookworms, as the intestinal bleeding caused by these parasites can seriously affect the volume studies during periods of slow absorption. The animals were treated with tetrachloroethylene to remove these worms and were then placed on a diet of lean meat, bread and milk, and kept under observation for at least a week in order to be certain that their condition as regards nutrition, etc., was satisfactory. Diarrhea is especially to be avoided. In the case of approximately 30 dogs which had developed this complaint it was found that no fluid whatever was absorbed, even at positive intraintestinal pressures. In fact in the case of all unhealthy animals used the condition of the intestine seems to have been affected so that in spite of the most careful handling the mucosa became inflamed and absorption ceased very early in the experiment. In such animals the normal blanching reaction of the mucosa on light mechanical stimulation is lost. The merest touch suffices to call forth a flush, and bleeding points may appear in the area within a few seconds. As it may require from 4 to 8 hours to obtain sufficient data for the plotting of absorption curves it is essential that every precaution be taken to provide animals in good condition.

In most instances the animals had fasted for 12 hours or longer before the operation. Water, however, was provided at all times. Sodium barbital was used as anesthetic in intravenous doses of 0.35 gram per kilo. In the operation a loop of jejunum, usually in a position just distal to the jejunal flexure, is brought out through a mid-line incision. The mesentery is slit radially between two arterial arches, allowing a tight ligation of the gut loop at one end. Twilled cotton tape, $\frac{1}{4}$ inch wide, is used in making all ligations, for it does not cut through the muscle. A slit 2 cm. in length is made through the wall near the ligature. The wire coil is then slipped into the lumen and carefully worked into position. Ligatures are then placed around the ends of the loop over the grooves in the rubber sleeves of the coil. These are tied as tightly as possible. The loop is then completely isolated by tying and cutting away the remaining gut, which is replaced in the abdomen, with the exception of the free ends, which are allowed to stay outside to avoid excessive infection of the peritoneal cavity.

The isolated loop is supported in a horizontal position outside the abdominal cavity by allowing it to rest on a horseshoe-shaped piece of thin board. The mesentery of the loop hangs free without tension on its vessels. The gut loop being outside the abdominal cavity the walls remain at atmospheric pressure, a condition which is conducive to the maintenance of a constant pressure difference on the two sides of the intestinal wall.

The whole animal is then placed in a metal box, containing water at 35°C. for a depth of one or more inches. The cover of this box is provided with a removable glass window and suitable holes for entrance of the rubber tubing. A cannula is placed in the trachea and connected to a short piece of rubber tubing in such a manner that the dog breathes the cool air of the outside room. Warm water is added to the box from time to time so that the temperature of the air inside remains at 35°C. Under these conditions it has been found that the rectal temperature of most animals will be maintained, without panting, at the normal of approximately 39°C. Use of such a box provides a warm, moist atmosphere for the isolated loop. Attempts to perform the experiment with the gut exposed at room temperature have led to irregular results, for as a result of efforts to maintain the gut in a moist condition it becomes chilled by evaporation of the fluid poured over it. Violent peristalsis is set up which changes the level of fluid in the burette in a totally irregular manner.

The loop is connected to the pipette or burette used in absorption rate determinations and to a stock bottle containing the solution to be used, which is maintained at body temperature. The loop is first rinsed out and filled. The tubing at the open end of the loop and that leading to the solution bottle are then clamped off with hemostats, leaving the connection between loop and pipette open. By means of a pair of levelling bulbs the position of the level of the upper surface of the board supporting the loop can be accurately determined in relation to a centimeter scale which occupies a vertical position close to the pipette. This level is arbitrarily considered as the atmospheric pressure or "zero pressure" level.

The interference due to peristalsis is one of the greatest difficulties to be surmounted in attempting to determine accurately the rate of absorption, especially at positive pressures. At negative pressures the wall of the gut is held firmly against the wire coil by the pressure of the atmosphere, and in most experiments the peristaltic waves have not caused fluctuations of the fluid level greater than 0.05 to 0.10 cc. As absorption proceeds the pipette is moved up a distance corresponding to 0.1 cc. after the absorption of each 0.1 cc. of fluid. In this way the pressure is maintained approximately constant as the volume decreases. The burette is read by the aid of an attached reading glass.

A stopwatch is used to measure the time for absorption of each 0.05, 0.10 or 0.50 cc. (depending on the speed of absorption). The successive time

intervals are tabulated during the progress of the experiment, and only such determinations are considered accurate in which variations between successive readings are regularly distributed about the mean. Observations of the rate are usually continued for at least an hour at each pressure. In all cases where absorption becomes progressively slower over long periods of time it is found that the gut loop has become edematous or that hemorrhage has occurred from the mucosal surface. Many incomplete experiments have had to be discarded due to interruptions of this nature. On the other hand no experiment in which obvious damage to the gut or to the dog as a whole was absent has ever given results of a less regular nature than those here reported.

Definite solutions of glucose (4 per cent) or sodium chloride (0.9 per cent) have been used throughout. No differences in the absorption that might be attributed to the difference in solute effect have been observed. Distilled water was found to cause edema of the whole gut wall, including the mucosa, with a gradual retardation of the absorption rate. With stronger solutions (6 per cent and 8 per cent glucose) the peristalsis is so marked that accurate determination of the absorption rate is difficult, even at negative pressure.

Peristalsis interferes to a marked degree with the accuracy of absorption rate measurements at positive pressure. To avoid this difficulty three methods of attack have been tried. At first a wooden supporting shield for the outside of the loop was provided, similar to the apparatus described in the article by Hamburger (1896). This gives a definite support to the expanded gut and therefore provides an accurate "baseline" of volume, which will not be much affected by the tone of the muscle. However, circulatory disturbances are produced, as evidenced by the appearance of marked edema, which have led me to discard the apparatus. The second method consists in measuring the decrease in volume after allowing the gut to dilate to a relatively stable condition of "diastolic" tone. This method has given consistent results but the possibility remains that one is measuring not absorption but a volume change due to a gradual and continuous relaxation of the gut. To avoid this possibility of error the third method was adopted, in which readings of the volume were made at a negative pressure of -4 or -8 cm., which causes the gut to collapse against the wire coil and provides a definite baseline of volume. The burette is then raised until a positive intra-intestinal pressure is produced. This pressure is maintained for a definite time, usually 10 minutes. The change in volume noted after the pressure has been again reduced to the negative baseline is taken as representing the volume absorbed in the given time. This method has given excellent results with pressures up to 12 cm. Above this point and often at lower pressures, the gut becomes so edematous that the experiment must be terminated. Violent peristalsis may be set up as a result of the

stretching of the gut wall. Positive pressure measurements are, in general, much more difficult to make than determinations at negative pressure.

In order to provide a smooth transition between absorption rates at negative and positive pressures it is necessary that the area of the absorbing surface of the gut remain constant. This condition is not fulfilled when the wire coil is used inside the gut—the area at negative pressure being only a fraction of that available when the gut wall expands away from the coil at positive pressure. A special clamp was therefore devised in which the gut could be stretched, with the intestinal wall in a horizontal plane. The loop is slit open longitudinally opposite the mesenteric border and the edges held in the clamp. The mesentery passes freely through a slot in the lower plate of the clamp. The upper piece of the clamp has an ellipsoidal opening which acts as a chamber to contain the solution. A glass cover is screwed in place, making the chamber water tight and allowing observation of the condition of the mucosa. Inlet and outlet tubes allow the proper connections to be made with the burette, and make it possible to rinse the chamber from time to time. A tray, consisting essentially of aluminum cross wires spaced 6 mm. apart, rests upon the surface of the mucosa. When a negative pressure is applied to the contents of the chamber the gut is pushed up against this grid. The wall thus receives adequate support without the surface for absorption being decreased to an appreciable extent. Such a clamp is difficult to apply and its use has resulted in only two experiments (out of approximately 30 attempts) in which absorption continued for a long enough time to enable the curve to be accurately plotted.

EXPERIMENTAL. The results of 11 of the 14 complete experiments that have been performed are represented graphically. The data which have been omitted would have caused confusion due to a too close overlapping of the curves. In two cases parts of the results of single experiments have been repeated in more than one graph.

Figure 2, plotted from data of 4 experiments chosen at random, illustrates the relation between intra-intestinal pressure and rate of absorption at negative pressures. The curves may be taken as representing a linear relationship, in spite of an occasional point which may lie at some distance from the line joining all other points. The reason for these occasional discrepancies is not clear. They seem to be related to periods of vigorous peristalsis, during which the determination of the absorption rate is usually difficult and unreliable. Extrapolation of the lines to zero rate indicates that absorption would cease at pressures from -16 to -26 cm. That such extrapolation is indeed justifiable is shown in figure 3. In these experiments the pressure was lowered far enough to cause an increase in volume of the fluid in the loop. These rates of increase in volume (called, for convenience, "negative absorption" rates) at different pressures allow ex-

trapolation to zero rate in the opposite direction. It is seen that the two extrapolated curves for each experiment meet the base line at approximately the same point.

It would be of interest to study the composition of the fluid "sucked" into the gut at the extreme low pressures. The fluid is not bloody and does not result from permanent damage to the gut, for in a number of instances determinations of positive and negative absorption were carried out alternately, without evidence of damage to the absorbing mechanism becoming evident. In this connection it is of interest to note that Hamburger (1896) in commenting on the failure to obtain an increase in volume of the gut contents at a pressure of −8 cm. remarked on the absence of any evidence of secretion of intestinal juice under these conditions. For this study he used a loop filled with air. Had he carried the pressure still lower there is no doubt that he would have obtained fluid.

Figure 4 presents the results of a few studies to determine the absorption rates at positive and negative pressures on the same animal, using the wire coil support for negative pressures. Above atmospheric pressure the expansion of the gut wall away from the coil allows a much more rapid absorption to take place. The points for positive pressure seem to lie along straight lines. Extrapolation of these lines to zero rate carries the (dotted) lines to the region of negative pressure, but, curiously enough, not as far as to the actual points at which absorption ceases. I cannot at present offer any definite explanation of this failure of the extrapolations of the positive and negative pressure curves to intersect at zero rate. It is possible that the absorbing surface becomes progressively greater as the gut expands under pressure, thus giving rise to an absorption curve whose shape is abnormally steep. One can only conclude that positive pressure measurements will not suffice for the calculation of the negative pressure which will cause absorption to cease. It is therefore fortunate that the accurate measurement of absorption rate is more easily carried out at negative pressure.

Two experiments are illustrated in figure 5 which show that, when the area of the absorbing surface of the mucosa is kept constant, the curve of absorption rate against pressure is a continuous straight line, without the break at atmospheric pressure which characterizes the curves of figure 4. These two experiments are the only ones which were carried to a successful termination in a large number of attempts to obtain data by the use of the special intestinal clamp described above. The clamp produces so much trauma to the gut tissue, as evidenced by the gradual appearance of bleeding points on the mucosal surface, that it is exceedingly difficult to study absorption by this method over the long periods of time necessary for the complete plotting of an absorption curve.

Discussion. It becomes evident from a study of the absorption curves

that the intestine, under the conditions of these particular experiments, exerts a definite absorbing force, a measure of which can be obtained by reducing the hydrostatic pressure of the contents of the loop to a point which just abolishes absorption. This force remains remarkably constant in each animal during the period of 4 to 8 hours necessary for the collection of the data. The negative pressure necessary to prevent absorption varies, as might be expected, in different animals, the range of pressures observed being from −8 to −26 cm. of solution. In addition it is noted that the curves, representing absorption rate plotted against pressure, are straight lines, so that the rate may be considered to increase in direct proportion to the increase in hydrostatic pressure, up to and above the level of atmospheric pressure.

The simplicity of the relationship between pressure and rate can be most easily explained by assuming that the absorption of fluid from the intestine occurs mainly as the result of the interaction of relatively simple physical factors. One is tempted to conclude that osmotic forces may suffice to explain the whole process. For, if we assume that on one side of a membrane there is a colloidal solution, whose colloid particles cannot pass through the membrane, while on the other side we have a solution of crystalloid whose solute particles pass the membrane with ease, there will occur a complete "absorption" of the crystalloid solution. To prevent this absorption one must either apply a positive pressure to the colloid solution or a negative pressure to the crystalloid solution. In either case the excess of hydrostatic pressure on the side of the colloid is a measure of the colloid osmotic pressure—the absorbing force. Furthermore, as the counterbalancing pressure difference is reduced the rate of osmosis will increase in proportion. The analogy to the behavior of fluid in the intestine under the particular experimental conditions is complete. We may therefore conclude that Starling's suggestion that the colloid osmotic pressure of the tissue fluid of the villi may be responsible for absorption is justified by the data so far obtained. It must be emphasized, however, that there are certain logical deductions from such a general theory of absorption which must be subjected to experimental test.

It is well known that an animal may absorb fluid from blood serum placed in the intestine. This fact has frequently been offered as the chief objection to any physical theory of absorption. However, it is well to point out that such experiments have never been properly controlled. Granting that the colloid osmotic pressure of the serum should prevent its absorption, there remains the probability, which has never been ruled out, that the serum undergoes changes on coming in contact with the intestinal mucosa and the digestive enzymes. Controlled experiments should include observations on the colloid osmotic pressure of serum remaining in the intestine at the end of the absorption period. The same criticism applies to

experiments on the absorption of gum acacia solution from the intestine (Rabinovitch, 1927). It will be necessary to reinvestigate these findings.

One must determine whether or not the normal contents of the gut exert a colloid osmotic pressure. Among the normal contents should be included the bile, the pancreatic and gastric juices, as well as the mixed contents containing the various common food constituents and their products of digestion.

The recent work of Drinker and his colleagues (1931) indicates clearly that the lymph is no different in composition from the tissue fluids. Consequently a study of the colloid osmotic pressure of the lymph from the lacteals should be made in order to determine whether this pressure corresponds to the absorbing pressure of the gut, as measured by absorption studies at negative pressure. The protein content of thoracic duct lymph, as determined by various workers (Drinker, 1931) seems to give evidence that the osmotic pressure of the tissue fluids of the villi may well be of the same order of magnitude as the counterbalancing negative pressures observed in my experiments.

Finally, it should be emphasized that although entirely abnormal, or at least unphysiological, pressure changes have been produced in these experiments, there is every reason to believe that a further study of the various osmotic and hydrostatic factors which operate under physiological conditions will throw considerable light not only on the absorption of fluids from the intestine but also on the more general problems of the relation between absorption and secretion, and of the physiological regulatory mechanisms which serve to maintain the normal balance in the exchange of fluid between the two sides of the intestinal wall. The results of a further investigation of the movements of fluid through the intestinal wall will, furthermore, contribute to a more thorough understanding of the factors which govern the absorption, secretion and excretion of substances dissolved in these fluids; for the direction and rate of movement of water through a membrane necessarily exerts a marked influence on the direction and rate of passage of substances in solution.

It has seemed advisable to suggest the above mentioned possibilities inherent in an osmotic theory of absorption in order that the logical corollaries of such a theory may be tested. The theory as a whole must stand or fall on the results of such tests.

SUMMARY

1. Water is absorbed from solutions of glucose or isotonic sodium chloride, introduced into isolated loops of the jejunum of the dog, at a rate which is constant at constant hydrostatic pressure.

2. The intra-intestinal pressure may be lowered to a value considerably below that of the atmosphere before absorption ceases.

3. The negative pressure, just sufficient to abolish absorption, has a constant value for each individual animal, and is therefore an exact measure of the absorbing force of the intestine. The values found in different animals have varied from -8 to -26 cm. of solution.

4. The rate of absorption of water is exactly proportional to the increase in intra-intestinal pressure above that negative pressure which just abolishes absorption.

5. These relationships may be expressed in the form of the absorption equation:

$$y = k\,(x - b)$$

where y is the rate, k is the constant of proportionality, x is the intra-intestinal pressure, and b is a constant which is measured by the pressure that abolishes absorption.

6. The simplicity of the above relationships indicates that absorption of fluid may be attributable to simple physical factors.

7. The suggestion of Starling that absorption of fluid is due to the colloid osmotic pressure of the tissue fluids of the villi seems to explain all the observed facts, and is accepted as a working hypothesis.

8. Some possibilities with regard to the regulation of the water exchange between the gut and the tissues, which are suggested by the osmotic theory of absorption, are considered in relation to the need for further study of these problems.

BIBLIOGRAPHY

DRINKER, C. K. AND M. E. FIELD. 1931. This Journal, xcvii, 32.
HAMBURGER, H. J. 1896. Arch. f. Anat. u. Physiol. (Physiol. Abt.), 428.
RABINOVITCH, J. 1927. This Journal, lxxxii, 279.
STARLING, E. H. 1898. Shäfer's Textbook of physiology, i, 291.
1920. Principles of human physiology, 3rd ed., 782.

THE EFFECT OF A DIET OF POLISHED RICE ON THE MINERAL CONTENT OF THE CARCASSES OF PIGEONS[1, 2]

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Contribution from the Department of Chemistry of the Kentucky Agricultural Experiment Station

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Recent investigations show that vitamin B contains as many as three different factors, B-1, the antineuritic factor, B-2, the antipellagric factor, and B-3, the factors responsible for increase in body weight.

The most pronounced example of vitamin B-1 and B-3 deficiency can be produced experimentally by feeding polished rice to pigeons. The purpose of the experiments described in this paper was to ascertain whether or not a diminution in one or more of the mineral constituents of the ash of the carcasses of pigeons would occur when they are brought down with experimental polyneuritis as the result of feeding polished rice.

In polishing rice the very thin, brown pericarp and germ are eroded away by a special kind of friction machine. Although the amount of material removed from the grains is very small, the composition of the pericarp is such that analyses of the polished and unpolished grains show that some rather marked alterations in chemical composition are brought about in the process of polishing. The results of the analyses of the polished and unpolished rice are contained in table 1.

The analyses show that the unpolished rice contains more than four times as much ash as the polished rice. The percentages of copper, zinc, sodium, nitrogen, sulfur and fat have not been seriously diminished as a result of polishing. The percentages of manganese, iron, and phosphorus, however, have been reduced to a little less than half, and those of calcium, magnesium and potassium to about a third of the corresponding percentages in the unpolished rice.

The following experiments were performed to ascertain what changes take place in the mineral constituents of the ash of the carcasses of pigeons when they are fed polished rice until polyneuritis develops.

[1] The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the Director.

[2] Presented before the Division of Agricultural and Food Chemistry of the American Chemical Society held at Indianapolis, March 30–April 4, 1931.

Lot 1 were normal pigeons which were chloroformed, the intestinal tract removed, the carcasses dried at 100°C., and ground for chemical analysis.

Polished rice was fed as the only diet to lot 2 until polyneuritis developed. The pigeons were then chloroformed and their carcasses prepared for analysis as in lot 1. In lot 3 the pigeons were brought down with polyneuritis in the same way as lot 2 but were then fed unpolished rice until they regained their normal weight, when they were chloroformed and their carcasses prepared for analysis as in lot 1. The results of careful and detailed chemical analyses of the ash of the three lots of pigeons are contained in table 2.

It is to be observed from the results contained in table 2 that the pigeons which were brought down with polyneuritis by feeding polished rice lost approximately one-third of their normal body weight in the meantime. That this loss was due principally to metabolized flesh is indicated by the larger percentages of ash, and smaller percentages of phosphorus and potassium in the ash of the pigeons. The pigeons which were brought down with polyneuritis regained their normal body weight on unpolished rice but did not make a complete recovery of phosphorus in the ash of their carcasses. The potassium content, however, was restored to nearly a normal condition in the ash of these pigeons.

TABLE 1

Analyses of polished and unpolished rice, in per cent of the moisture-free material

	POLISHED RICE	UNPOLISHED RICE		POLISHED RICE	UNPOLISHED RICE
Ash	0.42	1.90	Phosphorus	0.104	0.222
Copper	0.0003	0.0003	Sodium	0.060	0.063
Manganese	0.0013	0.0028	Potassium	0.073	0.261
Zinc	0.0028	0.0030	Nitrogen	1.37	1.41
Iron	0.0032	0.0076	Protein	8.56	8.81
Calcium	0.028	0.079	Sulfur	0.257	0.304
Magnesium	0.047	0.192	Ether extract (fat)	4.46	4.37

The percentage of calcium in the ash of the polyneuritic pigeons was a unit and seven-tenths larger than the normal, whereas in the cured pigeons it was slightly less. The percentage of phosphorus in the polyneuritic and restored birds was one and seventy-five-hundredths units and three and four-tenths units smaller, respectively than the normal. This fact suggests a loss of mineral matter during the time that the pigeons were receiving only polished rice. The percentage of potassium in the polyneuritic pigeons was distinctly less than in the normal birds but nearly the same as in the normal pigeons in lot 3.

In lot 4 the pigeons were fed polished rice until polyneuritis developed, after which each pigeon was fed a different cereal to ascertain if these grains

TABLE 2

Analyses of the ash of pigeons. Per cent

	LOT 1. FOUR NORMAL MALES					LOT 2. THREE MALES AFTER POLYNEURITIS HAD BEEN DEVELOPED				LOT 3. THREE MALES CURED OF POLYNEURITIS WITH UNPOLISHED RICE			
	1	2	3	4	Average	1	2	3	Average	1	2	3	Average
Copper (Cu)	0.022	0.006	0.010	0.009	0.012	0.012	0.010	0.013	0.012	0.009	0.010	0.011	0.010
Manganese (Mn)	0.004	0.002	0.006	0.003	0.004	0.002	0.006	0.002	0.003	0.003	0.002	0.003	0.003
Zinc (Zn)	0.018	0.025	0.023	0.024	0.023	0.012	0.045	0.115	0.057	0.054	0.083	0.091	0.076
Iron (Fe)	0.278	0.178	0.270	0.391	0.279	0.217	0.229	0.260	0.235	0.317	0.217	0.283	0.272
Calcium (Ca)	27.90	29.22	28.40	26.85	28.09	32.41	28.02	29.05	29.82	26.48	25.90	30.68	27.69
Magnesium (Mg)	0.796	1.38	1.36	1.34	1.22	0.665	1.61	1.36	1.211	2.00	1.59	1.82	1.80
Phosphorus (P)	18.80	16.82	17.92	17.34	17.72	17.58	14.70	15.56	15.94	13.08	13.11	16.89	14.36
Sodium (Na)	3.16	3.36	3.39	3.61	3.38	2.79	2.74	3.29	2.94	3.07	2.86	3.43	3.12
Potassium (K)	6.92	5.31	6.56	6.85	6.41	4.37	4.20	3.60	4.06	6.01	6.63	5.42	6.02
Weight of the dried carcass in grams	105.02	98.86	110.28	102.84	104.25	66.11	84.14	70.88	73.71	111.30	122.39	90.74	108.14
Per cent of ash in the dried carcass	10.42	11.35	11.89	11.77	11.36	13.82	14.83	14.98	14.54	9.79	9.69	10.24	9.91

possessed the property of curing polyneuritis and restoring the loss of body weight. The results obtained with wheat, barley, rye, unpolished rice, white corn and yellow corn are shown in the graph. It is to be observed that wheat, unpolished rice and yellow corn possessed the property of curing polyneuritis and restoring the body weight to a normal state. Yellow corn accomplished this more slowly than wheat or unpolished rice. This

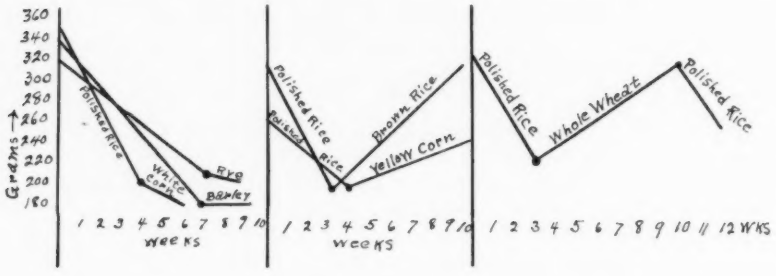

TABLE 3

Analyses of barley, white corn, yellow corn, rye, wheat and unpolished rice, in per cent of the moisture-free material

	BARLEY	WHITE CORN	RYE	YELLOW CORN	WHEAT	UNPOLISHED RICE
Ash	3.23	1.49	2.14	2.08	1.78	1.90
Copper	0.0004	0.0003	0.0003	0.0004	0.0008	0.0003
Manganese	0.0019	0.0008	0.0030	0.0010	0.0060	0.0028
Zinc	0.0057	0.0048	0.0044	0.0048	0.0043	0.003
Iron	0.0053	0.0034	0.0067	0.0053	0.0078	0.0076
Calcium	0.061	0.021	0.098	0.021	0.063	0.079
Magnesium	0.173	0.156	0.196	0.150	0.168	0.192
Phosphorus	0.467	0.298	0.391	0.380	0.351	0.222
Sodium	0.254	0.117	0.200	0.123	0.193	0.063
Potassium	0.660	0.208	0.463	0.465	0.456	0.261
Silica (SiO_2)	0.720	0.067	0.085	0.074	0.077	
Nitrogen	1.90	1.80	2.19	1.75	2.15	1.41
Protein (N × 6.25)	11.88	11.25	13.70	10.95	13.45	8.81
Moisture in air-dry material	8.29	8.74	8.28	8.02	9.66	

fact shows that whole wheat, unpolished rice and yellow corn contain the B-1 and B-3 vitamin factors, although yellow corn apparently contains less of these factors than wheat or unpolished rice.

A complete analysis of the ash of each of these cereals was made. The results, calculated to the moisture-free condition, are contained in table 3.

From table 3 it will be observed that the cereals which cured the pigeons and produced normal gains in body weight contain slightly larger quanti-

ties of copper, manganese and iron than the cereals which did not effect any gain in body weight. This observation suggested further experiments in which pigeons were brought down with polyneuritis and small quantities of inorganic salts of manganese, copper, zinc, iron and various mixtures of these were injected into the blood stream of the afflicted pigeons. Four males and 2 females were given injections of the following: One male was given intraveneous injections daily of 1 cc. of a solution containing 0.0001 gram Mn; one male was treated likewise with a solution containing 0.0001 gram Zn per cc.; another was treated with 0.0001 gram Mn and 0.0001 gram Zn daily; and the fourth male was injected with 0.0001 gram each of Cu, Mn and Zn. The two females were treated likewise, one with 0.0001 gram Cu and 0.0001 gram Mn. The other with Cu + Mn + Zn. No permanent relief was obtained with any of the elements injected.

DISCUSSION OF THE RESULTS. Beccari (1) has found that potassium exerts a stimulating effect on the nerves and increases muscular contractibility when fed in the form of inorganic salts. Miller (2) discovered that growth of young rats was greatly retarded by reducing the potassium content of the diet to less than one-tenth of one per cent, although no other marked abnormality was observed. He states that inadequate potassium in the diet during the very early development of the animals not only prevented growth of body tissues but caused abnormal physical changes, such as respiratory trouble, and edema of the eyes. Moreover, when potassium was added to the diet, growth resumed rapidly.

Osborne and Mendel (3) in a series of metabolism experiments stressed the importance of potassium in the diet and the inability of sodium to replace potassium functionally.

SUMMARY

In polishing brown rice, substances containing considerable quantities of ash, manganese, iron, calcium, magnesium, phosphorus and potassium are removed in the offal. When pigeons are fed polished rice until polyneuritis develops, analyses of the ash of their carcasses show marked diminution in magnesium, phosphorus and potassium content as well as in the content of some of the minor constituents.

Unpolished rice, wheat and yellow corn cured polyneuritis and restored normal body weight, showing that they contained vitamin B-1 and B-3 factors, though yellow corn produced slower recovery and gain in body weight than unpolished rice and wheat. Barley, rye and white corn contained vitamin B-1 but were deficient in the vitamin B-3 factor. Wheat, unpolished rice and yellow corn contained more of the elements copper, manganese and iron than barley, rye and white corn, which leads the authors to infer that the minor mineral constituents as well as the major constituents calcium, magnesium, phosphorus and potassium, are factors in protecting pigeons from polyneuritis.

BIBLIOGRAPHY

(1) BECCARI, L. Arch. Ital. Biol., lxiii, 321; Zentralbl. Biochem. Biophys., xx, 335; Chem. Abs., 1919, xiii, 3227. Original not seen.
(2) MILLER, H. G. Journ. Biol. Chem., 1923, lv, 61.
(3) OSBORNE, T. B. AND L. B. MENDEL. Journ. Biol. Chem., 1918, xxxiv, 135.

A STUDY OF SPONTANEOUS ACTIVITY IN THE EXCISED UTERINE HORNS OF THE RAT, BY AN IMPROVED METHOD

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Since the work of Kehrer (1) the method for immersing uterine strips and oxygenating the immersion medium has not changed appreciably. Smith and McClosky (2) and Pittenger (3) have offered elaborate apparatus but have not improved upon the original in principle. Using the method of Kehrer (1) or Laidlaw and Dale (4), Blair (5) studied the contraction rate of the rat's uterus and reported upon the relation of 2 types of contraction (maximal and submaximal) to the events of oestrus. Clark, Knaus and Parkes (6) using essentially the same method found no certain relation between frequency of contraction and the stages of the oestrous cycle.

It is evident that in studies upon excised uterine preparations by these methods, where the normal spontaneous activity is recorded, wide variations in activity exist, while if the tissue is stimulated or depressed by some powerful agent a more constant response occurs. This is certainly true in biological standardization experiments.

In the present paper a study is made of the normal spontaneous contractions of the excised uterus of the adult virgin albino rat kept under controlled conditions. The spontaneous muscular contractions evoked are studied by an improved method, and considered from 3 aspects: 1, the types of contractions; 2, activity as related to the oestrous cycle, and 3, reaction to certain experimental treatment.

Apparatus. The apparatus used for this work (see fig. 1) consists essentially of 3 units. *1*, A muscle chamber, *m*, of 50 cc. capacity; *2*, a reservoir *R*, of 100 cc. capacity to facilitate refilling of the muscle chamber, and *3*, a pump *O*, which provides a means for oxygenating and gently circulating the immersion medium. Pyrex glass is used throughout and the immersion medium is Locke's solution.

The muscle chamber is provided with an overflow vent *C*, and an L-tube *A*, for fractional evacuation. It communicates with the reservoir by means of a stout capillary tube (1 mm. bore) on the course of which is a three-way stopcock *2*, through which the solution from the reservoir is passed to the muscle chamber or discarded.

The pump—a system of tubes (3 mm. bore) communicates with the

muscle chamber at 2 points, *1*, the bottom where it takes its charge, and *2*, at the level of the Locke's solution where it discharges. It is provided at its upper end with an expansion bulb *G*, which acts as a safety reservoir during violent pump action, and a side limb *L*, (1 mm. bore) through which oxygen is admitted.

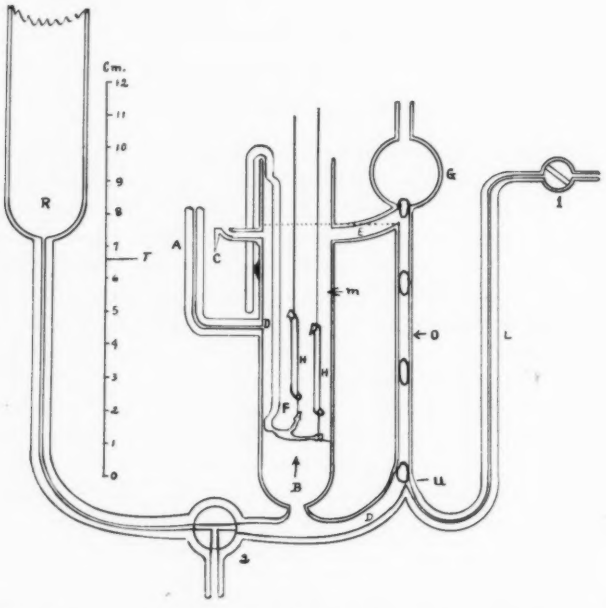

Fig. 1. Showing apparatus used in the study of excised uterus. *m*, muscle chamber; *R*, reservoir in which solutions are placed, preparatory to refilling muscle chamber; *O*, pump; *1*, stopcock for regulating oxygen flow; *L*, capillary tube (1 mm. bore) through which oxygen passes to pump; *U*, point at which oxygen bubbles form; *G*, expansion reservoir; *C*, overflow vent; *A*, exhaust tube for fractional evacuation of chamber; *2*, three-way stopcock; *B*, direction of fluid when pump is not operating; *F*, removable foot; *H*, represents uterine horns suspended between foot, and writing levers; *T*, level to which the whole apparatus is immersed in a constant temperature bath at 37°C.; *D*, outlet of L-tube; *E*, communication between muscle chamber and top of pump; *D*, communication between bottom of muscle chamber and pump; scale in centimeters.

The removable foot *F*, is an independent unit made to rest by a hook upon the chamber wall, and acts as the anchoring point for the tissues.

Oxygen is supplied from a tank, through a diaphragm valve at 2 pounds pressure. It is washed through a 1 per cent aqueous solution of sodium bicarbonate, which is renewed at 3 hour intervals. The degree of agitation of the solution is determined by the rate of flow of oxygen.

In this apparatus two experimental difficulties have been overcome: 1. the circulation and oxygenation of the Locke's solution is accomplished without disturbing the immersed tissue; 2, the immersion medium of the muscle chamber can be changed without exposing the tissues therein to the air.

Manipulation and preparation of the apparatus. The apparatus is supported by a clamp fixed to a stand, and all immersed in a constant temperature bath at 37°C., to within 1 cm. of the overflow vent *C*. The muscle chamber and reservoir are filled with fresh Locke's solution, care being exercised that stopcock *2* is in a neutral position. The oxygen flow is adjusted at stopcock *1* to about 2 bubbles per second. In 6 minutes the temperature, amount of dissolved oxygen and pH should be constant.

To effect a change of the solution in the muscle chamber without exposing the tissues to the air, 3 steps are necessary: 1, With a filter pump or other suitable means aspirate fluid through L-tube *A* to level *D*, (this does not expose the tissues); 2, with the aspirator still operating slowly pass Locke's solution in through stopcock *2*, until at least 50 cc. have been used; 3, discontinue the exhaust allowing the muscle chamber to slowly re-fill to overflowing. Continue the flushing with Locke's solution until all traces of the previous solution have disappeared. This usually consumes roughly 100 cc. of Locke's.

Variations of this procedure produce equally good results, so long as the principles are adhered to.

LOCKE'S SOLUTION. Formula:

Sodium chloride c.p.	9.000 grams
Potassium chloride c.p.	0.420 gram
Calcium chloride $2H_2O$	0.240 gram
Dextrose, anhydrous powder	0.500 gram
Sodium bicarbonate q.s. pH 8.0 about	0.500 gram
Double distilled water q.s.	1000 cc. 20°C.

This solution is prepared daily and kept at room temperature. The pH is adjusted to 8.0 at 20°C. with sodium bicarbonate. When oxygenated the pH drops to 7.7, where it remains within narrow limits for 3 hours. The specific conductivity is 1.6×10^{-2} reciprocal ohms at 25°C. The amount of dissolved oxygen varies slightly but does not affect noticeably the activity of the tissues so long as it is kept above 0.03 cc. per 100 cc. of Locke's. The method of oxygenation herein described offers a wide margin of safety—the solution showing an average of 0.035 cc. of oxygen per 100 cc. of Locke's. (Determinations made by the Winkler method.)

ANIMAL MATERIAL. The rats used in these experiments were selected for age, weight, and condition. Animals 90 days old, weighing 120 grams each (plus or minus 5 gm.) and in good condition were grouped as follows: 1, litter mates from our own stock; 2, litter mates from 2 outside sources;

3, individuals from our own and outside stocks. These were segregated and placed upon our standard diet,[1] which was kept constantly before them. These groups represented our source of presumably normal animals. The certainty of their normal condition was ascertained by daily or even hourly vaginal smears following the technic of Long and Evans (7). A satisfactory repetition of 4 or more complete cycles as demonstrated by this smear method was the criterion by which the animal was adjudged sexually normal. Only those so selected are reported in this paper.

METHOD. The animal is selected, and weighed. A final vaginal smear is made. The animal is then killed by a blow on the head, the abdominal

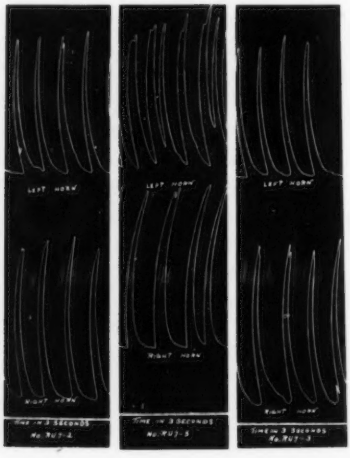

Fig. 2. Showing close similarity of maximal contractions in stage III. Three different animals are represented, with records from both horns made simultaneously. The responses of the right horns are always higher than the left, due to the slight difference in length. The right horns are longer.

wall is opened, the horns are quickly cut free of their attachments and removed to oxygenated Locke's solution at room temperature. Here one end of each horn is immediately attached by thread to the removable foot of the apparatus, while to the opposite ends are tied long threads for attachment to the recording levers. The horns are then quickly transferred to the muscle chamber and the free threads connected with the recording lever which should exert a pull of from $\frac{1}{2}$ to 1 gram. By so adjusting the pull of the levers no detrimental effects result, and it does facilitate recording barely perceptible responses.

[1] Whole wheat 64.5; casein 15.0; klim 10.0; sodium chloride 1.0; calcium carbonate 1.5; butter 8.0, supplemented by whole milk and leafy vegetables on alternate days.

By this method paired horns from a given animal respond so nearly alike as to appear identical, and if horns from different animals in exactly the same stage of the oestrous cycle are used they likewise respond similarly, both in rate and amplitude of contraction. (See fig. 2.)

Not only is similarity of response shown by this method, but also differences even though slight. Whole horns excised at different stages of the oestrous cycle react quite differently, and if a uterine horn be divided into segments and these permitted to record their individual activity, we find that under certain conditions the segments respond similarly, while under others they differ widely. Thus in stage III the segments react with remarkable similarity, while in no other stage is this true. On the contrary in all the other stages the tubal end responds like a whole horn, while

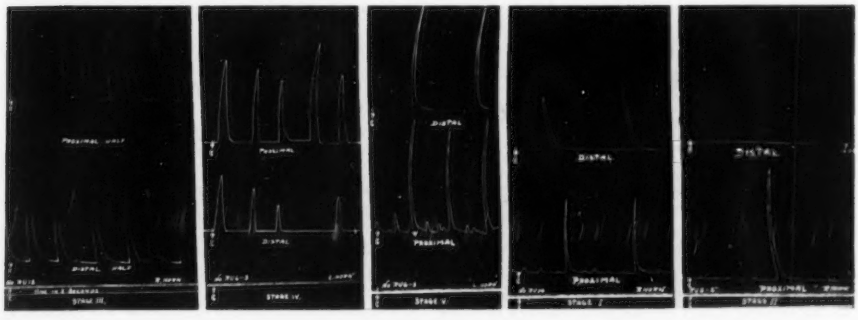

Fig. 3. Showing the activity of proximal and distal halves of uterine horns, as they occur in different stages of the oestrous cycle. Note the absence of submaximal contractions in the distal ends.

the vaginal end always shows a slower rate of contraction and seldom exhibits the more delicate responses. (See fig. 3.)

Types of contractions. With the animal material of this series and using the method just described, we found 3 types of contractions elicited by the uterus (see fig. 4). (A 4th type appears elsewhere, but is outside the scope of this paper.) These types are:

1. Maximal contractions: these are complete powerful responses, involving the entire segment or horn. They are evoked by all excised tissue of this series, whether it be an entire horn or only a segment. The frequency with which they occur varies with the stage of the oestrous cycle. (See *1*, fig. 5.)

2. Submaximal responses: those having all the major characteristics of maximal contractions, but which vary in amplitude from a mere beat to maximum height. (See *2*, fig. 5.) They, too, are characteristic responses of this series, but are practically annihilated in stages I and II, and completely over-shadowed by maximal contractions in stage III.

3. Circular contractions: these are poorly recorded by this method but are reflected upon the record as barely perceptible responses, having a slight amplitude and a broad peak. (See *3*, fig. 5.) They are easily confused with submaximal contractions approaching obliteration, but can be identified by experimental treatment, which will be described presently.

4. Composite contractions: in this type one contraction is superimposed upon the other. (See *4*, fig. 4.) They are irregular and are not observed

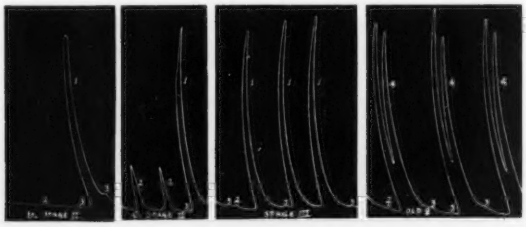

Fig. 4. Showing the 4 types of spontaneous contractions of the uterus: *1*, maximal; *2*, submaximal; *3*, circular; *4*, composite.

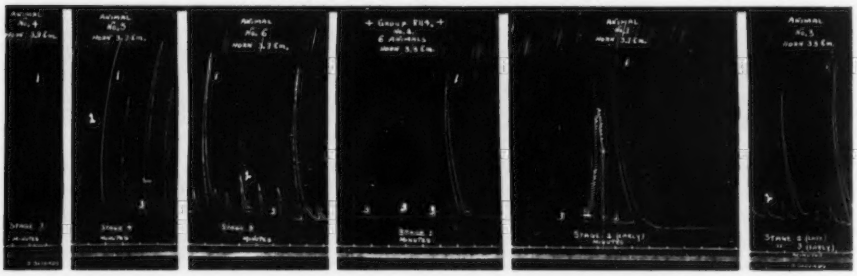

Fig. 5. Showing types of contractions in different stages of the oestrous cycle of the rat. *1*, Maximal, involving entire horn. All stages. *2*, Submaximal, involving principally the tubal end. Stages IV, V, and late II. *3*, Reflection of circular responses. Stages I and II.

Note. Composite responses seldom appear in the sexually active uterus. (See fig. 2.)

in the responses of this series. (A study of this contraction is now in progress.)

If attention be now directed to the relation of maximal and submaximal contractions to the different phases of the oestrous cycle (see fig. 5), as determined by the vaginal smears, it will be observed that, traced successively from stage III the maximal contractions are replaced in an orderly manner by submaximal contractions throughout the remainder of the cycle, and that the latter progressively dwindle and finally disappear.

Thus, stage IV shows alternate maximal and strong submaximal contractions. In stage V a further alternating group of maximal contractions have dropped out and the substituting submaximal contractions have dwindled in size. In stage I the maximal contractions have reached a minimum in number while the submaximal contractions have completely disappeared. In stage II (early) the maximal contractions are as in the preceding stage, but there is a feeble evidence of returning submaximal contractions. This picture in stage II (late) or early stage III is replaced by well developed submaximal contractions interposed between still widey separated maximal responses; one submaximal contraction having,

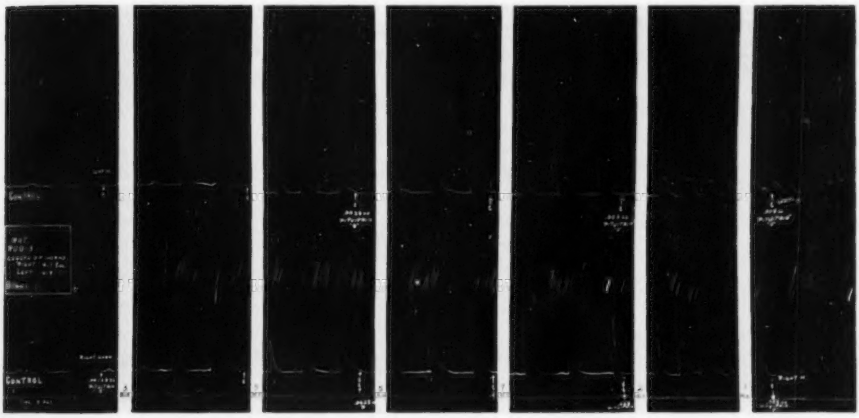

Fig. 6. Showing the reaction of 2 uterine horns of an animal in stage I to small doses of pituitrin "0." The section of the record marked *Control* is a small strip but represents the type of response over a 1-hour period. In this time the ratio of 2 submaximal to 1 maximal contraction had not changed in either horn. Note that after all contractions become maximal, further addition of pituitrin affects both rate and tonus.

however, attained almost the size of the maximal contraction. Further amplification of growth of these submaximal contractions leads directly to the pattern as seen in stage III and to a completion of the cycle. Thus, submaximal contractions may be interpreted as phases of obliteration or of restoration of maximal contractions in the process of rhythmic slowing or accelerating of the uterine muscle, this rhythm following faithfully the rhythmic cycle as shown by vaginal smears.

The reaction of submaximal responses to pituitrin. If the above described phenomena have been correctly interpreted it should be possible by appropriate artificial stimulation to transform the quiescent uterus of stage I into the active state or stage III, thus reversing the "dropping out" process of

submaximal contractions. This was done. The uteri of animals in stage I were prepared in the usual manner, and to the bath were added minute doses (0.00125 to 0.005 cc.) of pituitrin "O" at intervals of 10 to 15 min-

TABLE 1

Results obtained when animals were selected for the experiment by the vaginal smear method

ANIMAL NUMBER	WEIGHT	STAGE	FINDINGS—SMEAR	LENGTH OF HORN	REMARKS	WORK
	grams			*cm.*		*gram-cm.*
2	164	I	Epithelial cells	4.0	Mid-stage	14.8
1	168	II	Few cornified cells	4.2	Oestrus	12.0
11	164	Ea. III	Many cornified cells	3.9	Late oestrus	55.5
3	168	III	Much cornified material	4.0	Mid-stage	75.3
5	162	IV	Cornified cells, epithelial cells and leucocytes	3.9		62.5
7	162	V	Leucocytes and cornified cells	3.7	Early V	36.0
6	164	V	Leucocytes only	3.9	Mid-stage	40.0
12	164	V	Leucocytes and few epithelial cells	3.8	Late V	24.0

TABLE 2

Results obtained when animals were killed at approximately 10-hour intervals over entire cycle

ANIMAL NUMBER	WEIGHT	HOURS AFTER OESTRUS	LENGTH OF HORN	WORK	VAGINAL SMEAR—FINDINGS BEFORE ANIMAL WAS KILLED	REMARKS
	grams		*cm.*	*gram-cm.*		
2	164	Oestrus	4.0	14.0	Cornified cells	Oestrus
13	165	16	3.9	27.5	Cornified cells	Oestrus
4	162	25	3.7	75.6	Cornified cells	Late oestrus
14	168	35	4.1	43.2	Cornified and leucocytes	Early V
19	165	45	3.9	22.5	Leucocytes	Stage V
17	170	58	3.9	22.0	Leucocytes	Stage V
24	165	65	3.8	22.0	Leucocytes	Stage V
16	172	76	3.9	18.0	Leucocytes	Stage V
21	164	85	4.0	14.0	Leucocytes and few epithelial cells	Late V
22	170	95	4.2	10.0	Epithelial cells	Stage I

utes, until all the submaximal contractions became maximal. (See fig. 6.) In this manner the restorative phase of the cycle is duplicated. What part pituitrin plays in this process as it occurs in life we cannot say. In this

instance it serves to confirm the assumption that the contraction types as observed at various stages of the oestrous cycle are the result of varying degrees of activity of the uterine musculature, and can be interpreted as above outlined.

The curve of activity of the oestrous cycle. From the standpoint of activity of the uterine musculature there seem to be no divisions according to stages, but rather a gradual transition, producing a wave of activity for the entire cycle, the phases of which coincide with the oestrual stages as shown by the cell pictures of the vaginal smears (table 1). The least active stage (early stage II) will be seen to immediately precede the most active stage (stage III), which in turn is succeeded by an intermediate stage of activity progressively diminishing through stages IV, V, and I, toward the point of least activity, (stage II early) but never becoming entirely inactive. This wave of activity in this series faithfully repeats with each cycle.

The results presented in table 1 were checked by a second series of experiments based upon chronological data. The animals were killed at intervals of 10 hours throughout the cycle, all calculations being made from oestrus (table 2). The results obtained are expressed in gram-centimeters of work per hour, thus establishing confirmation by a method quite independent of that based upon the oestrual stages.

Curves constructed from these tables favorably coincide with each other.

We feel that either of the above methods will result in the establishment of a curve of activity for the oestrous cycle that will closely represent at any period of the cycle the spontaneous activity of the excised structure for that moment.

CONCLUSIONS

1. An improved method for handling the excised uterus of the rat is presented, and new apparatus described.

2. The rhythmic variations of the uterine contractions are due to the gradual suppression of maximal contractions, with their subsequent restoration, the contraction type conforming to the so-called stages of the oestrous cycle.

3. The peak of muscular activity occurs in stage III.

4. In this series the activity of the uterus ranged from 10 gm.-cm. of work in stage II to 75 gm.-cm. in stage III.

5. Faintly perceptible responses are transformed into maximal contractions by the use of minute doses of pituitrin.

Acknowledgments. I wish to express my appreciation to Dr. Carl L. Davis for his council in the preparation of this paper, and to Dr. Carlson for his kind review of the manuscript.

BIBLIOGRAPHY

(1) KEHRER: Arch. f. Gynäk., 1907, lxxxi, 160.
(2) SMITH AND MCCLOSKY: Hyg. Lab. Bull., 1924, cxxxviii, 1.
(3) PITTENGER: Amer. Journ. Pharm., 1927, xcix, 531.
(4) DALE AND LAIDLAW: Journ. Pharm. Exper. Therap., 1912, iv, 75.
(5) BLAIR: This Journal, 1923, lxv, 223.
(6) CLARK, KNAUS AND PARKES: Journ. Pharm. Exper. Therap., 1926, xxvi, 359.
(7) LONG AND EVANS: Memoirs Univ. California, 6, Univ. of Cal. Press, 1922.

STUDIES ON THE FLOW OF BILE INTO THE DUODENUM AND THE EXISTENCE OF A SPHINCTER OF ODDI

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Although numerous studies have been made on the mechanism controlling the flow of bile into the duodenum, the literature is not in agreement. It is agreed anatomically that muscle fibers pass about the intramural portion of the common duct in such a manner as to exert a sphincteric action and the majority of anatomical investigations agree that there is a special constrictor about the orifice of the papilla; but the existence of a special sphincter has been questioned and even denied by those who regard muscular tonus of the duodenum as the sole controlling factor. The literature up to 1928 is reviewed by Babkin (1928).

It occurred to the author that by studying the "pressure gradient" in the intramural portion of the common duct with a special device for ascertaining pressures at various levels, one might be able to determine the relative rôle played by the duodenal fibers surrounding the duct and prove or disprove the existence of a sphincter at the ampulla. Further, since no one has recorded graphically and simultaneously by adequate methods the pressure relations between the common duct and duodenal musculature under various conditions (Copher and Kodama, 1926), such as changes in duodenal motility and tone under nerve and drug stimulation, it was decided to make such a study with the hope that an absence or presence of a correlation in the pressure relationships might provide decisive data.

METHODS. In all experiments anesthetized large dogs (18–22 kilos) were used. Since trauma to or incision of the duodenum would probably alter the results, the duodenum was not incised in any of the experiments and was manipulated as little as possible, except in three experiments to be described later.

For recording duodenal tone and motility a condom balloon was used which was approximately two inches long. The balloon was inserted into the duodenum through a slit in the stomach near the pylorus and passed down to the level of the intramural portion of the common bile duct. A ligature at the site of the incision in the stomach held the balloon in place. The balloon was connected to a water manometer for recording peristalsis

and pressure levels. Only a few cubic centimeters (3–5) of air were placed in the balloon in order to avoid overdistention of the duodenum.

For recording the resistance offered to the flow of fluid into the duodenum, the apparatus used was an adaptation of that of Elman and McMaster (1926). The inflow in drops was recorded on the smoked drum by observing the dripping bulb. In order to measure and record the pressure on the drum it was necessary to use bromoform in the manometer (sp. gr. 2.65). An appropriately flexed glass cannula was inserted into the extramural portion of the duct about 4 cm. above its entrance into the duodenum and connected to the perfusing fluid in the large Trendelenburg jar. This jar could be easily lowered and raised to or set at any desired pressure level and was connected with the bromoform manometer. Ringer's solution

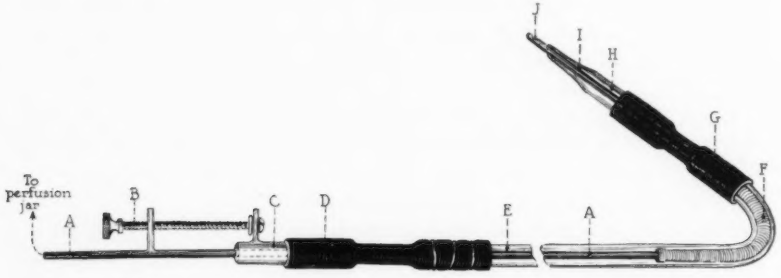

Fig. 1. The figure shows the special device used for studying the intramural "pressure gradient." The inner system through which the Ringer's solution passed consists of brass tube, *A*, connected with the stiff rubber tube, *F*, which is connected with the brass cannula, *I*. The outer system consists of brass tube, *C*, rubber pressure tubing, *D*, glass tubing, *E*, rubber pressure tubing, *G*, and glass cannula to be tied into the extramural portion of the common duct. By means of the thumb screw the inner system could be moved within the outer system. *J* is the orifice of the brass cannula which is protected by a nipple of solder.

was the fluid used in all experiments and the temperature was maintained at 38.0°C. by means of a thermostat and water jacket.

For recording and measuring the intramural "pressure gradient" in the duct, a special apparatus was used (fig. 1). The apparatus is made up essentially of an outer and inner tube system, the outer system being fixed and the inner system through which the Ringer's solution flows being movable inside the outer. This device consisted of an outer glass tube 4 mm. in diameter appropriately flexed at the distal end to join a large glass cannula placed in the duct. Inside of the straight portion of the glass tube a brass tube 2 mm. in diameter was placed. At the flexed portion of the outer glass tube a stiff rubber tube 3 mm. in diameter was connected to the brass tube. Inside the glass cannula in the duct was placed a brass cannula

1.5 mm. in diameter with a smooth rounded end, outlet on the side at the end, which was connected with the stiff rubber tube. This inner system of brass tube, stiff rubber tube and brass cannula was attached to a thumb screw device. This made it possible to push the brass cannula at will through the glass cannula into the duct and down millimeter by millimeter into the intramural portion of the duct and out through the ampulla into the lumen of the duodenum and back again, measuring the pressure of inflow at the various levels.

RESULTS. The pressure necessary to cause fluid to flow from the duct into the duodenum will be referred to as the perfusion pressure or intramural resistance.

Since seventy-five dogs were used in this study, it is impractical to cite all experimental results in detail.

Anesthesia. In order to determine the most suitable anesthetic several preliminary experiments were performed. It was soon found that barbital (0.15–0.20 gm. per kilo) was the most suitable in that it interfered less with the tone and motility of the duodenum. However, it was necessary at times to use ether during the operative procedures after which it was withdrawn. Deep ether anesthesia abolished the tone of the duodenum and greatly reduced the perfusion pressure. Morphine increased the amplitude of the duodenal peristalsis and increased the perfusion pressure, and was used very effectively to study the relation between duodenal peristalsis and the perfusion pressure. Asphyxia increased markedly the intramural resistance and agumented duodenal tone and peristalsis. The effect of asphyxia was studied because of the blood pressure reducing effects of some of the drugs to be used.

Mechanical effects. Early in each experiment considerable care had to be exercised to avoid artificial pressure due to mechanical occlusion. However, one soon learns to detect and avoid this complication.

It was necessary to study the effect of the amount of air in the balloon on the perfusion pressure. It was found that as the pressure in the balloon was increased and decreased, corresponding changes in perfusion pressure resulted; but the balloon had to be distended considerably in order to materially influence the perfusion pressure. Nevertheless, only that amount of air was placed in the balloon which did not affect perfusion pressure, which amounted generally to about 5 cc. of air. This was tested in each experiment.

Initial perfusion pressure or intramural resistance. In the series of 75 dogs the average initial pressure was 160 mm. H_2O. The greatest number varied between 110 and 135, the maximum being 590 and the minimum 65 mm. H_2O.

Effect of vagus on intramural resistance. Section of the right vagus resulted in a decrease in intramural resistance in 10 out of 17 dogs, no

change in 6 and an increase in 1. The intraduodenal pressure showed no change in 11, was increased in 1 and decreased in 5. The increase in duodenal pressure was accompanied by an increase in intramural resistance. In one case a decrease in duodenal pressure was accompanied by a decrease in intramural resistance. Section of the left vagus after the right usually was without effect. A further decrease in resistance without change in the duodenum was noted in one case (fig. 2, D). Irrespective of how the

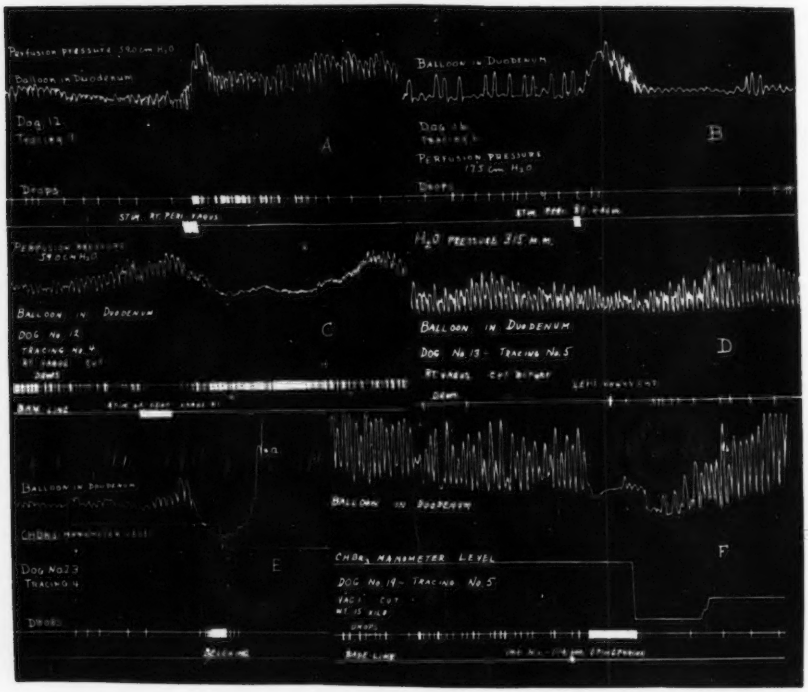

Fig. 2. This figure shows the effect of vagus section, *d*, and stimulation, A, B, C, on duodenal tone and intramural resistance. A tracing showing the effect of belching and epinephrine is included.

resistance was affected, it generally returned to normal within ten minutes. An analysis of the data on the initial intramural resistance and duodenal tone and the variable response to sectioning of the vagi shows no correlation. This statement holds true for vagus stimulation also.

Stimulation of the right vagus peripherally with the left vagus intact resulted in a decrease in intramural resistance in 5 and an increase in 5 dogs (fig. 2, A and B). The intraduodenal pressure was increased in 6, decreased

in 2, and no change occurred in 2. In two dogs an increase in duodenal pressure was accompanied by a decrease in intramural pressure; but in none did a decrease in duodenal pressure accompany increase in intramural resistance.

Stimulation of the right vagus centrally with left vagus intact decreased the intramural resistance in 4, and increased it in 1, and no change occurred in 2 dogs (fig. 2, C). The intraduodenal pressure was increased in 1, decreased in 3, and no change occurred in 3. In one case the intramural resistance decreased and the duodenal pressure increased. After both vagi were cut, stimulation of the right vagus peripherally caused only an increase in resistance and stimulation of the central end was usually ineffective, although a decrease was noted in a few instances.

The reader may wonder why the results in each dog are not analyzed. This was done, but did not throw further light on the situation, because the response varies in the same dog from time to time. For example, in one of the ten dogs, stimulation of the right vagus peripherally on one occasion caused an increase in duodenal tone with an increase in intramural resistance, and then later it caused an increase in duodenal tone with a decrease in intramural resistance. The following points are quite evident from our results: *a*, the right vagus exercises a tonic motor control over the musculature surrounding the intramural portion of the common bile duct, irrespective of changes in the duodenal musculature; *b*, stimulation of the right vagus peripherally may increase or decrease intramural resistance irrespective of duodenal changes (fig. 2, A and B); *c*, stimulation of the right vagus centrally usually causes a decrease in intramural resistance. The most important point is that the changes in intramural resistance do not always follow the changes in intraduodenal pressure.

Belching. Since it is so unusual for the dog to belch and since no one has ever had a balloon in the duodenum during the act of belching, it is worth while to record the changes that occurred in one instance. In dog 23 unexplained changes in the pressures were noted and then gas was eructated from the stomach. A study of the tracing (fig. 2, E) shows that early in the act, intraduodenal pressure decreased as did the intramural resistance or perfusion pressure. Later there occurred a marked contraction of the duodenum with a cessation of flow from the duct into the duodenum. Part of the marked rise in duodenal pressure is attributable to skeletal contraction but not all (fig. 2, E, *a*).

The relation of duodenal motility to intramural resistance. Early in this work it was observed in the dogs which showed good spontaneous duodenal peristaltic activity that the intramural resistance varied with the peristaltic movements and that the flow from the duct into the duodenum was intermittent. This could be demonstrated in most dogs after exciting peristaltic activity with morphine. This was best demonstrated graphically

on the tracings by raising the pressure level to the point at which only a small amount of inflow was recorded. The resulting tracings (fig. 3) showed unquestionably that the inflow was usually intermittent and synchronized with the duodenal peristalses. During the contraction no flow into the duodenum occurred but during relaxation inflow occurred.

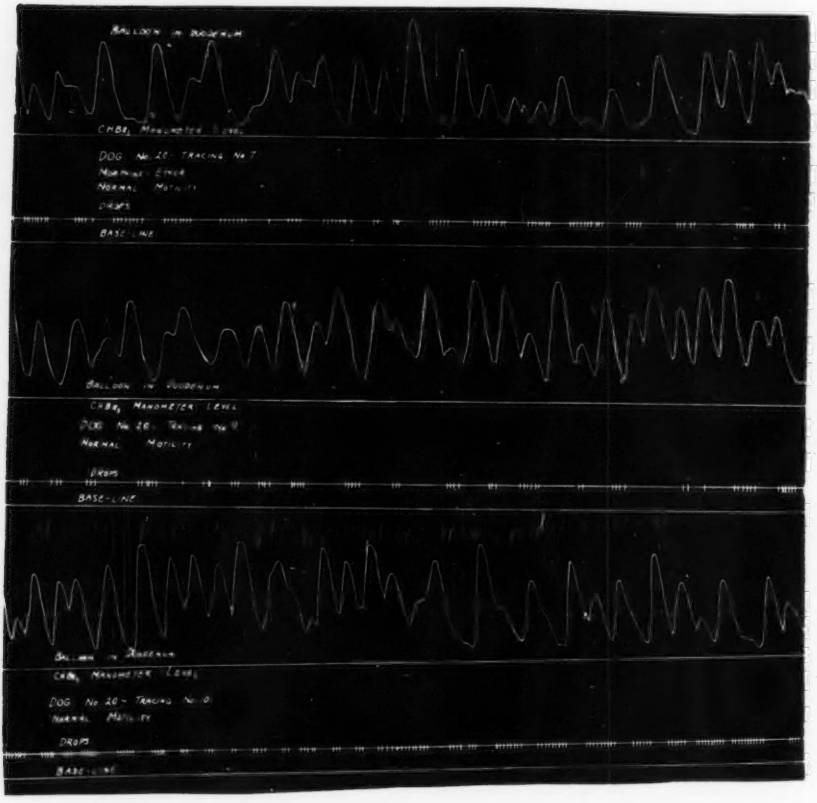

Fig. 3. This figure shows the relation between duodenal peristalses or contractions and inflow from the common duct into the duodenum.

This phenomenon was not an artifact due to the balloon because it occurred whether or not the balloon was inflated or in place. The correlation between relaxation and decreased resistance to flow was not always as perfect as demonstrated in the tracings shown in figure 3. The decreased resistance to flow was sometimes observed early in relaxation and at other times late just preceding the subsequent contraction of the duodenum.

Occasionally contraction and relaxation of the duodenum would occur without any decrease in intramural resistance. Only rarely did we observe a flow during a contraction, and on examination of the position of the balloon we found it to be above or below the intramural portion of the duct. This explains the fluctuating resistance to the flow of bile in the intestine observed by Elman and McMaster (1926) when their dogs were fed. In the course of these experiments on 20 dogs we as a rule found no changes in intramural resistance independent of duodenal contractions or changes in duodenal tone as recorded by the balloon. This shows that the sphincteric mechanism is intimately correlated with duodenal activity.

Is the intramural resistance equivalent to the tone or the pressure exerted by the duodenal musculature? It occurred to us that a study of the relation of the amount of pressure exerted by the duodenal musculature on the balloon to the amount of pressure necessary to cause fluid to pass from the duct into the duodenum might throw some light on the existence of a special sphincter at the orifice of the papilla.

In making this study the initial pressure of the duodenum on the balloon and the initial pressure necessary to cause fluid to flow into the duodenum were determined. Then pilocarpine (5 mgm.) was injected. This caused a cessation of flow into the duodenum and a massive contraction of the duodenum (fig. 4). The rise in pressure of the duodenum was measured and then the amount of pressure required to reëstablish flow into the duodenum. This was done in 12 dogs. It was found in all but two dogs that the additional pressure required to force fluid into the duodenum was greater than the rise in duodenal pressure. The difference amounted approximately to from 20 to 60 mm. H_2O usually. In dog 33, it amounted to 100 mm. H_2O (fig. 4). This was also true when the duodenum manifested spontaneously a temporary state of hypertonus. This suggested that a sphincteric mechanism other than duodenal musculature is present and was caused to contract by the pilocarpine. However, the balloon in the duodenum records, so far as is known, only or chiefly the contraction of the circular muscles. Since the common bile duct passes through the longitudinal muscle layer of the duodenum, the contraction of these muscles may compress the duct and therefore may be responsible for the difference in the pressures noted. There is also reason to doubt that a balloon with only 5 to 10 cc. of air in it accurately records total pressure exerted by a contraction. So these results are interesting, but not decisive.

The intramural "pressure gradient". It was postulated that if the duodenal musculature exerted pressure on the intramural portion of the common bile duct, and if there was a sphincter about the orifice of the ampulla, this could be shown by determining the "pressure gradient" or the intramural resistance to the flow of bile as various levels in the common bile duct.

In three preliminary experiments a thin walled glass cannula 3 cm. long and 1.2 mm. in diameter slightly rounded and open at the end was inserted through a cut in the common duct pointing toward the duodenum. The cannula was connected to the perfusion pressure recording system. Prior to this, large dogs were anesthetized with barbital and the stomach and

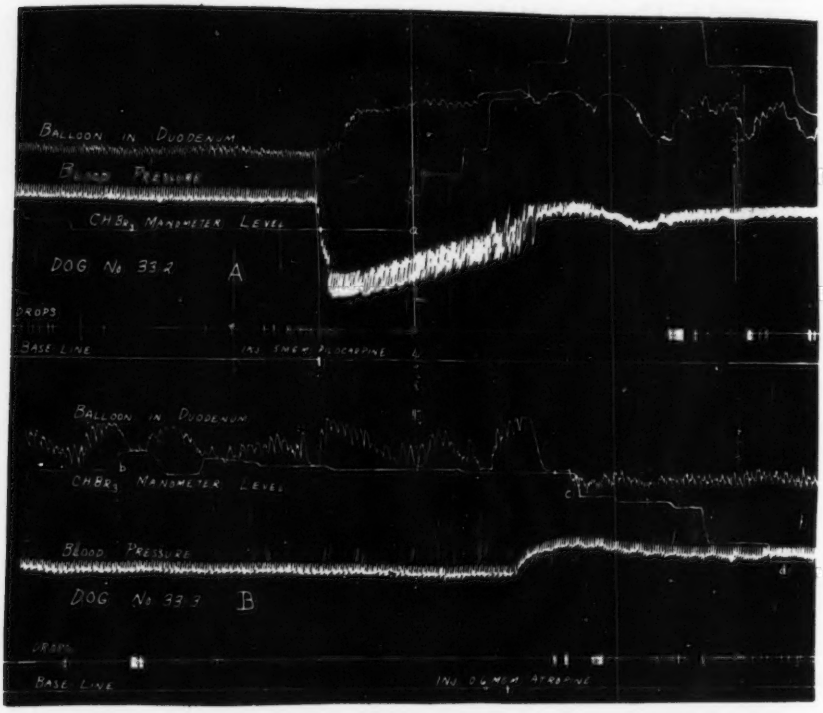

Fig. 4. This figure shows the relative effects of pilocarpine and atropine on intraduodenal pressure and intramural resistance. At *a* the Trendelenberg jar was raised, increasing the perfusion pressure until a flow was reëstablished at the end of tracing A. On tracing B at *b* the jar was raised and lowered to ascertain how much pressure the duodenal variations in tone were exerting on the intramural portion of the duct. At *c* the jar was lowered to ascertain the amount of change in intramural resistance caused by atropine.

duodenum mobilized by sectioning the common duct and gastro-hepatic ligament. This made it possible to lift the pyloric antrum and duodenum out and rendered the insertion and manipulation of the cannula easy and accurate. The duodenum was then slit opposite the ampulla. It was found that the resistance to flow fell as the glass cannula was moved down-

ward which was done at steps of from 2 to 3 mm. When the end of the cannula reached a point in the duct 1 to 2 mm. proximal to the orifice of the papilla, it could be readily seen through the mucosa and the resistance at this point was regarded as the resistance of the sphincter of the orifice. The reading of the pressure required to cause a flow was recorded and then the cannula was moved gently through the orifice into the duodenum and a check of the perfusion pressure for purposes of making hydrostatic corrections of pressure was made. Then the cannula was drawn back by stages into the extramural portion of the duct.

In these experiments it was found *a*, that the ampulla could be seen to bulge with fluid before the orifice opened; *b*, that the resistance decreased as the cannula passed down the duct and increased as the cannula passed up the duct (fig. 5, lower tracing); *c*, that the resistance when the cannula was from one to two millimeters from the orifice of the papilla varied from 6 to 13 cm. of water pressure in different dogs and from 3 to 5 cm. in the same dog from time to time; *d*, that the total resistance of the intramural portion of the common duct varied by this method from 30 to 50 cm. of water, and *e*, that if a large dog is used and the manipulations are gentle and properly performed, trauma to the orifice of the ampulla can be avoided.

Realizing that incision of the duodenum is not a normal procedure, we next devised the special apparatus (fig. 1) described above for measuring the intramural pressure gradient without injuring the gastro-hepatic structures and the duodenum, and at the same time recording the intraduodenal pressure with a balloon. The apparatus was tried out in several dogs with the duodenum slit as in the preceding experiments and then after each experiment the brass cannula and distances it moved was checked as far as possible, which, of course, was only approximately correct. In moving the cannula down the duct the first time, the way into the duodenum had to be "felt" so to speak as described in the legend of figure 5.

Experiments were performed on 10 dogs as follows: with the special cannula in place in the extramural portion of the common duct the total perfusion pressure was obtained. Then the cannula was moved by stages down the duct, the perfusion pressure being recorded on the tracing at each stage, until the cannula entered the duodenum. The cannula was moved downward slowly so as to prevent it from kinking the duct and from exciting a local spasm mechanically. The cannula was then withdrawn in stages. As the cannula descended the intramural portion of the duct, the resistance decreased in a step-like manner and fell either to or almost to zero on entering the lumen of the duodenum. On withdrawing the cannula a step-like rise in resistance occurred until the cannula reached its original position outside the wall of the duodenum. After the descent and ascent of the cannula the initial perfusion pressure was rarely obtained

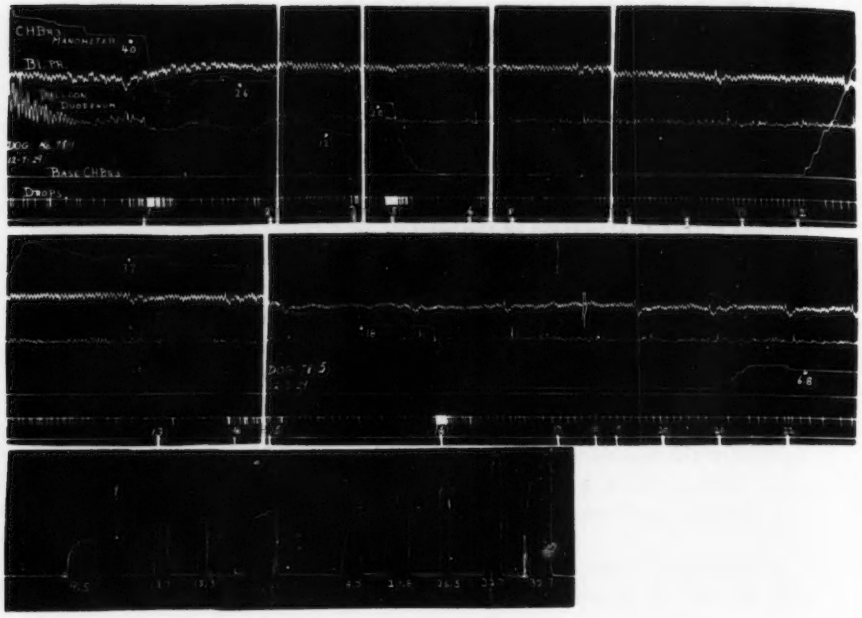

Fig. 5. This figure (upper two tracings) shows sections of tracings obtained on dog 78, in which the device for the determination of the intramural "pressure gradient" was used. The pressure extramurally was 40.8 cm. H_2O. At *1* the brass cannula was moved downward approximately 1 cm. in the duct. The pressure to reëstablish normal flow was 26.5 cm. At *2* the cannula was moved down another centimeter. The pressure was approximately 12.7 cm. We believe we were near the orifice of the papilla, but of course were not certain. At *3* the cannula was drawn upward about 0.5 cm. The pressure (20.1 cm.) rose about to the midpoint of the last two pressures. At *5* the cannula was moved downward a little more than 0.5 cm., and the pressure fell to almost zero, or the perfusion pressure of the open system as later determined. Decreasing the pressure slightly stopped the flow. It was raised again a little and the flow was reëstablished at *6* and *8*. At *9*, *10*, and *11*, the cannula was drawn upward for three or four turns of the thumb screw. No change of pressure or flow resulted, which convinced us that the end of the brass cannula was in the duodenum. At *12* the cannula was drawn upward approximately 2.0 cm. and the pressure extramurally, just prior to *13* was 37.6 cm. At *13*, *14* and *15* the cannula was moved downward about 1.5 cm., the pressure necessary to reëstablish "normal" flow being about 18 cm. At *16* the cannula was moved downward about 0.5 cm., and evidently passed into the duodenum. At *17*, *18*, *19* and *20* the thumb screw was turned each time one turn or so. At *21* the flow stopped and the pressure was raised and the "normal" flow established just beyond *22*. We believe that at *21* the cannula had been drawn upward into the ampulla. The pressure was corrected by 1.5 cm. H_2O because when the cannula was evidently in the duodenum the manometer level did not fall to zero. We accepted 6.8 cm. H_2O as the resistance offered by the sphincter at the orifice of the papilla.

suggesting that some stretching—no contraction or edema—of the mechanism causing the resistance had occurred. On repeating the procedure a number of times in the same dog, the step-like change could be obtained every time. The resistance was decreased but not abolished.

We were particularly interested in observing the resistance just preceding the passage of the cannula into the duodenum, because we believed that the resistance offered at this point would indicate the existence of and be due to the action of a submucosal sphincter about the orifice of the ampulla. A resistance varying from 20 to 60 mm. H_2O was encountered. This resistance amounted to from one-third to one-sixth of the total intramural resistance.

In two instances the usual step-like fall in perfusion pressure was observed, and then just prior to the passage of the cannula into the duodenum a definite rise in pressure resulted which was maintained for a minute or so until the cannula was pushed into the duodenum. In these instances on withdrawal of the cannula from the duodenum the high pressure was again observed followed in a few minutes by a fall in pressure, then on further withdrawal of the cannula the usual step-like rise was observed. Several interpretations of this phenomenon are possible; it might represent either a localized spasm of an ampullar sphincter or a mechanical occlusion of the cannula. However, close examination of the cannula and observation of the duodenum for the presence of a spasm or contraction along with the definite rise in pressure on withdrawing the cannula convinced us that the most probable explanation of the phenomenon was that it was due to a spasm or contraction of a sphincter located at the ampullar orifice.

Effect of drugs. In many of the experiments the effect of various drugs on the intramural resistance was studied. All drugs were injected intravenously, except where otherwise stated.

Pilocarpine in doses from 1 to 5 mgm. generally caused a marked increase in intraduodenal pressure, and a prompt cessation of flow from the duct into the duodenum. It was necessary to markedly increase the perfusion pressure to reëstablish the flow. In three out of 23 cases the intraduodenal pressure was unchanged; in two of these there was a slight increase in perfusion pressure; and in the third a slight decrease in perfusion pressure.

The lower tracing was obtained with the duodenum opened and mobilized by section of the gastro-hepatic ligaments and a cannula at various levels in the intramural portion of the common duct. A pressure of 9.5 cm. H_2O was necessary to open the sphincter of the papilla when the end of the cannula was from 1 to 1.5 mm. from it; 13.7 cm. when 2 to 3 mm. distant; 15.3 cm. at 3 to 4 mm. Beyond this we were not certain, but approximated that the pressure of 17.4 cm. was obtained at 5 mm.; 18.5 cm. at 6–7 mm. and 23.8 cm. at 8–9 mm. The first pressure of 32.7 cm. was obtained when the cannula was about 4 mm. distal to the junction of the common duct with the external wall of the duodenum. The second pressure of 32.7 was obtained with the cannula at the junction of the duct with the duodenal wall.

Atropine quite uniformly caused a reduction of intraduodenal pressure and a decrease in perfusion pressure when given in doses of from 0.4 to 1.5 mgm. Atropine in adequate dosage counteracted the effect of pilocarpine. In two out of 23 dogs, no change in intraduodenal pressure occurred but the perfusion pressure was decreased.

Nicotine in doses of 2 to 5 mgm. abolished the regular duodenal contractions and reduced the tone of the duodenum. The intramural resistance was decidedly decreased (3 expts.). Later the duodenal tone gradually returned, but never returned to normal. The intramural resistance varied directly with the amount of recovery of the duodenal musculature.

Epinephrine when given in doses from from 1 to 5 cc. of a 1:10,000 dilution decreased duodenal pressure and the intramural resistance (fig. 2, F). With smaller doses (0.5–2.0 cc. of 1:100,000) the opposite effect was usually obtained, but the results were not consistent (19 expts.).

Ephedrine (2–10 mgm.) had the same effect as epinephrine, except in one dog (5 expts.) the effect was reversed.

Pituitrin and oxytocin usually caused (8 expts.) an initial decrease in intraduodenal pressure and intramural resistance followed by a slow return to the control level. In one dog the decrease in intramural resistance definitely preceded the fall in duodenal pressure.

Histamine (0.25–0.5 mgm.) produced (8 expts.) a rise in intraduodenal pressure and an equivalent rise in intramural resistance.

When acetylcholine was given (5 expts.) in doses to cause a considerable fall in blood pressure, its action paralleled that of histamine, although not as consistently. In two experiments the intramural resistance fell even though the duodenal pressure remained the same or increased.

Ergotamine tartrate (0.25–0.5 mgm.) usually caused (12 expts.) an initial increase in duodenal pressure with a subsequent decrease. The intramural resistance followed the changes in duodenal pressure.

Benzyl acetate and benzyl alcohol injected intravenously in doses sufficient to cause a slight fall in blood pressure did not influence duodenal pressure or the intramural resistance.

Caffeine, procaine and strychnine in otherwise effective doses caused no changes in the duodenal and intramural resistance.

Magnesium sulphate solutions were instilled directly into the duodenum just distal to the balloon by placing a small rubber tube into the duodenum at the time the balloon was put in place. When 5 cc. of a 25 per cent solution (39°C.) were slowly injected into the duodenum the intraduodenal pressure was slightly increased (5 expts.). The intramural resistance was decreased for 1 to 1½ minutes and then it returned to the control level. This effect was also obtained after the vagi were cut. Instillation of 20 cc. of the same solution resulted in either an increase or no change in intramural pressure.

It is to be noted that the effect of drugs on the intramural resistance parallels their effect on intraduodenal pressure with the exceptions noted. It should be added that as a general rule the increase in intramural resistance caused by the various drugs exceeded the increase in intraduodenal pressure as was mentioned before under the topic headed "Is the intramural resistance equivalent to the tone or pressure exerted by the duodenal musculature?"

Discussion. Oddi (1894) reported that stimulation of the vagus peripherally causes a strong contraction of the sphincter and claimed an independent nervous control for the sphincter. Westphal (1923), who divided the sphincteric mechanism into two portions—the duodenal portion and the sphincter of the papilla—reported that vagus stimulation causes the duodenal portion to contract and the sphincter to relax. Our data show that Oddi was partially correct because we found that peripheral vagus stimulation increased intramural resistance, although in an equal number of experiments it was decreased. In this connection it is noteworthy that only increased resistance occurred on vagus stimulation when both nerves were cut. The fact that intramural resistance may change irrespective of the changes in intraduodenal pressure proves the existence of an independent nervous control. In regard to the reciprocal innervation of the sphincter of the papilla and the duodenal portion of the sphincteric mechanism suggested by Westphal, our data do not prove it, neither do those of Westphal. In our two experiments in which vagus stimulation caused a rise in duodenal pressure and a decrease in intramural resistance, two interpretations are possible, first, that the sphincter of the papilla relaxed more than the duodenal portion contracted, second, that both portions of the mechanism relaxed. The observation that stimulation of the vagus peripherally caused an increase in resistance in five dogs and a decrease in five, caused us to consider the possibility that the sphincteric mechanism might be analogous to the pyloric sphincter in regard to response to nerve stimulation in that Carlson and Litt (1924) showed that vagus stimulation would cause relaxation of the contracted sphincter and contraction of the relaxed sphincter. However, when we studied the initial intramural resistance in regard to whether vagus stimulation caused a decrease or an increase in resistance and in regard to the state of duodenal tone we found no correlation. The existence of two variable factors, namely, initial resistance and duodenal tone, either one of which may be effected oppositely, presents a situation very difficult to analyze with certainty.

The frequency with which the intramural resistance decreases following section of the vagus, even without an evident change in duodenal tone in some cases, shows that the vagus exercises a tonic motor control over the sphincteric mechanism of the common duct. This is very probably the mechanism concerned in the flow of bile initiated according to some observ-

ers on the sight and smell and early after the ingestion of food (Elman and McMaster, 1926; Babkin, 1928; Boyden, 1927), but which does not materially affect the size of the gall bladder in sham feeding experiments in dogs (Crandall, 1931) and does not always occur (Krause and Whitaker, 1928).

That duodenal motility and tone play a rôle in controlling the flow of bile into the duodenum is strongly supported by the observations of a number of workers (Copher and Kodama, 1926; Elman and McMaster, 1926; Burget, 1926; Berg and Jobling, 1927; Brugsch and Horsters, 1926, and others). Our tracings graphically record this relationship and establish adequately that such a relationship exists. This does not mean, however, that a flow of bile into the intestine cannot occur without duodenal relaxation, because, as stated above, it does occur. These observations agree with those of Giordano and Mann (1927) who state that bile may spurt from the papillary orifice without discernible peristalsis, but if peristaltic waves are in the duodenum, the outflow of bile will not occur except with peristalsis.

The results obtained in answer to the question, "Is the intramural resistance equivalent to the tone or pressure exerted by the duodenal musculature on the balloon" showed that the intramural resistance was always greater than intraduodenal pressure. This indicated the existence of a special sphincteric mechanism at the orifice of the papilla. Although we questioned the decisiveness of these results for reasons enumerated before, it is striking that the difference in intramural resistance and intraduodenal pressure (20 to 60 mm. H_2O) is the same as the resistance offered by the sphincter of the papilla obtained by the special device for measuring the intramural "pressure gradient." This can hardly be a coincidence, since ten dogs were used in one series of experiments and twelve in the other. It could not be due to the presence of the balloon because of reasons enumerated in the first part of this paper.

Our observations and data on the intramural "pressure gradient" along with the evidence obtained by others (literature summarized by Giordano and Mann, 1927), convince us that the intramural portion of the common duct possesses a special sphincteric mechanism. That a special sphincter of the papilla exists is shown by the fact that when a cannula is placed in the intramural portion of the duct one or two millimeters proximal to the orifice of the papilla, or at the level of the submucosal layer, and pressure exerted on the fluid in the cannula, the ampulla can be seen to bulge and the pressure must be raised to an adequate threshold level before the fluid flows into the duodenum. That the adequate threshold level of pressure necessary to open the sphincter varies from 3 to 5 cm. in the same dog from time to time with the cannula in the same place shows that the tone of the sphincter varies. We know of no other reasonable way of interpreting these observations.

SUMMARY AND CONCLUSIONS

1. A tracing showing the changes in intraduodenal pressure and intramural resistance as influenced by belching was obtained.

2. The vagi exercise a tonic motor control over the musculature surrounding the intramural portion of the common bile dut, which may or may not be correlated with changes in tone of the duodenal musculature.

3. Stimulation of the peripheral vagus may increase or decrease intramural resistance irrespective of changes in duodenal tone. The two observations just stated confirm Oddi who claimed an independent nervous control for the sphincter. The usual effect of peripheral vagus stimulation is contraction of the duodenum and increased intramural resistance.

4. Inflow of bile into the duodenum is intimately correlated with duodenal peristalsis. Given a pressure just sufficient to overcome intramural resistance, and given active duodenal peristalsis, inflow into the duodenum occurs during relaxation but ceases during contraction of the duodenum. Exceptions to this generalization occur infrequently.

5. When the duodenum is caused to contract by pilocarpine or manifests spontaneously a marked hypertonus, the increase in intraduodenal pressure as registered by a balloon is from 20 to 60 mm. H_2O less as a rule than the increase in intraduct pressure necessary to reëstablish inflow into the duodenum.

6. An intramural "pressure gradient" exists in the common bile duct; that is, as a cannula is passed downward through the intramural portion of the duct, the amount of pressure required to cause a flow into the duodenum decreases, and when the movement of the cannula is reversed, the pressure increases.

7. A physiological sphincter is located about the orifice of the duct in the papilla in the duodenal mucosa. This sphincter exerts from $\frac{1}{3}$ to $\frac{1}{6}$ of the total resistance offered to the flow of fluid through the intramural portion of the common duct. Under the conditions of one set of experiments, the "sphincter of the orifice" was forced open by a pressure varying from 20 to 60 mm. H_2O; in another set of experiments with the duodenum slit, the pressure varied from 60 to 130 mm. H_2O.

8. The effects of pilocarpine, atropine, morphine, nicotine, epinephrine, ephedrine, pituitrin, histamine, ergotamine, benzyl acetate and alcohol, caffeine, procaine, and strychnine intravenously on the intramural resistance were studied. In general it was found that the effect of these drugs influences the intramural resistance and duodenal tone in a similar manner. Occasionally, however, the intramural resistance would undergo a decrease or increase without a change in duodenal tone. It is important that atropine may decrease the intramural resistance without effecting duodenal tone.

9. Magnesium sulphate solution (25 per cent) instilled into the duo-

denum in small amounts (5 cc.) usually decreases intramural resistance for a short period in spite of a gradual increase in duodenal tone. Larger amounts definitely augment duodenal tone and peristalsis and may increase or have no effect on intramural resistance.

10. We are convinced that the intramural portion of the common bile duct possesses a special sphincteric mechanism, that a sphincter of the papilla exists and that this sphincteric mechanism is intimately coördinated with duodenal peristalsis and tone, but may function independently of the duodenum. Our data do not permit us to conclude whether the portion of the sphincteric mechanism located in the duodenal musculature has a reciprocal relation with the sphincter of the papilla or whether it functions independently of the duodenal musculature.

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BIBLIOGRAPHY

Babkin. 1928. Aussere Sekretion der Verdauungsdrüsen. 2nd ed. Berlin.
Berg and Jobling. 1927. Proc. Soc. Exper. Biol. and Med., xxiv, 434.
Boyden. 1927. Proc. Soc. Exper. Biol. and Med., xxv, 99.
Brugsch and Horsters. 1926. Arch. f. exper. Path. u. Pharm., cxviii, 267.
Burget. 1926. This Journal, lxxix, 130.
Carlson and Litt. 1924. Arch. Int. Med., xxxiii, 281.
Copher. 1925. Journ. Amer. Med. Assoc., lxxxiv, 1563.
Copher and Kodama. 1926. Arch. Int. Med., xxxviii, 647.
Crain. 1931. Surg., Gynec. and Obstet., in press.
Crandall. 1931. Arch. Int. Med., in press.
Elman and McMaster. 1926. Journ. Exper. Med., xliv, 151.
Giordano and Mann. 1927. Arch. Path. and Lab. Med., iv, 943.
Ivy and Walsh. 1930. Ann. Int. Med., iv, 134.
Ivy and Oldberg. 1928. This Journal, lxxxvi, 599.
Krause and Whitaker. 1928. This Journal, lxxxvii, 172.
McMaster and Rous. 1921. Journ. Exper. Med., xxxiii, 731.
Oddi. 1894. Sperimentale, xlviii, 180, Abst. Schmidt's Jahrb., 1895, ccxlv, 120.
Westphal. 1923. Zeitschr. f. klin. Med., xcvi, 52.
Whitaker. 1926. This Journal, lxxviii, 411.
Winkelstein and Aschner. 1925. Amer. Journ. Med. Sci., clxix, 679.

THE POSTERIOR PITUITARY HORMONE IN METABOLISM

II. The Effect of Pitressin and Pituitrin Upon the Carbohydrate Reserves of Adrenalectomized Rabbits

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In an earlier paper (Bischoff and Long, 1931) the question was raised as to whether or not the production of hyperglycemia and hyperlactic acidemia in the rabbit following pitressin administration was due to the liberation of adrenalin from the adrenals. The possibility that the posterior pituitary hormone functions through the adrenal system has been repeatedly alluded to in the older literature. In order to answer this question, the following plan of experimentation with medullary and with totally adrenalectomized rabbits was undertaken.

1. Establishment of total or bilateral medullary adrenalectomy by the determination of the minimum convulsion-producing dose of insulin and the blood lactic acid curve at insulin hypoglycemia, and by section of any suspicious tissue at the termination of the experiments.

2. Determination of the blood sugar and blood lactic acid curves following the administration of pitressin or pituitrin with and without the simultaneous administration of minimum convulsion-producing doses of insulin.

3. Determination of the liver and muscle glycogen and muscle sugar of pituitrin dosed and control medullary adrenalectomized rabbits.

Experimental. *Adrenalectomy.* Three groups of rabbits were studied. The adrenalectomy was done in two stages, the technique recommended by Britton, Geiling and Calvery (1928) being used. The adrenal on the right was completely removed. In the first group three-fourths of the adrenal on the left was removed in the second operation. The second group consisted of totally adrenalectomized animals. In the third group approximately nine-tenths of the cortex was removed. Two of these animals showed signs of adrenal insufficiency following the operation, but eventually recovered, and were in excellent condition as were all the animals in the first group up to the day of sacrifice.

Methods. In excising tissue samples the animals were stunned in the

first and second series. Amytal anesthesia was used in the third se
The analytical procedures are the same as described in former papers. In our last series of experiments the reducing power of the muscle sugar extract was determined after fermentation. The changes in sampling and the addition of the fermentation procedure have been added to eliminate the objections raised to older methods.

RESULTS. *Minimum insulin convulsion-producing dose.* In the first series three of four medullary adrenalectomized rabbits convulsed after the administration of 0.5 unit of insulin per kilo given subcutaneously. One rabbit required 0.8 unit. Normal rabbits require from 3 to 7 units to produce convulsions. In the first series no attempt was made to establish the minimum convulsion-producing dose of insulin. In the third series three rabbits convulsed after the administration of 0.25 unit insulin per kilo. Two of the rabbits convulsed on several occasions after the administration of 0.15 unit per kilo. Some weeks later 0.20 unit was required.

Pitressin and pituitrin. Fifteen units of pitressin given subcutaneously produced a slight (30 to 70 mgm. rise per 100 cc.) hyperglycemia in five of the medullary adrenalectomized rabbits. A rise in blood lactic acid ranging from 11 to 47 mgm. per 100 cc. was noted in each case one-half hour after dosing. Normal values were observed at two and one-half hours. The results are no different than were observed when normal rabbits received the same dose of pitressin. In two rabbits both pitressin and pituitrin produced a slight but significant hypoglycemia and failed to abolish insulin convulsions, when the experiment was performed a month after the final operation. Several months later the same doses abolished insulin hypoglycemia completely. In the first series of medullary adrenalectomized rabbits, 15 units of pitressin given subcutaneously prevented insulin convulsion (0.5 unit insulin per kilo) in two cases and delayed the occurrence two hours in a third rabbit. In a fourth rabbit, which did not convulse when 0.5 unit of insulin was given, pitressin lessened the hypoglycemic curve. In one rabbit of group III, pitressin and pituitrin both abolished insulin hypoglycemia when administered a month after the operation. Rises in blood lactic acid, similar to those produced when pitressin was given alone, were sometimes observed. Protocols for only two of the seven rabbits studied are presented. They are representative of the results obtained for the entire group.

Blood lactic acid at insulin hypoglycemia. It is now generally conceded that during insulin hypoglycemia there is a liberation of adrenalin as a defense mechanism (Cori, 1931). Since minute quantities of adrenalin produce a lactic acidemia, and since insulin is without effect in abolishing such a lactic acidemia, a rise in blood lactic acid should be observed during insulin hypoglycemia. The earlier work of Briggs, Koechig, Doisy and

...r (1923), which indicated such an effect, was not confirmed by Best and Ridout (1925) and Cori (1925) for hypoglycemia without convulsions, although all workers agree that there is a rise in blood lactic acid following convulsions. The resting values for the blood lactic acid of rabbits obtained by Cori in 1925 (13.1–39.7) are higher than the values obtained in 1929 (7–24). While these differences may be relative, due to a difference

Rabbit 5

DATE	TREATMENT		BLOOD FINDINGS IN MG. PER 100 CC.								
			Initial	0.25 hour	0.5 hour	0.75 hour	1 hour	1.5 hours	2 hours	2.5 hours	3.5 hours
Feb. 25	15 u. pituitrin per kilo subcutaneously	Sugar	101	91	79		55		67		
		Lactic acid	15	30	30		30		17		
Mar. 2	15 u. pitressin per kilo subcutaneously	Sugar	108	103	85		82		77		
		Lactic acid	12	31	36		31		14		
Mar. 6	15 u. pituitrin, 0.3 u. insulin per kilo subcutaneously	Sugar	96	93	70		40	40*			
		Lactic acid	14	14	15		15	16			
Mar. 10	15 u. pitressin, 0.3 u. insulin per kilo subcutaneously	Sugar	87		†		52	41*			
		Lactic acid	5				11	11			
May 12	15 u. pituitrin, 0.3 u. insulin per kilo subcutaneously	Sugar	106		101†	103	114	100		83	
		Lactic acid	13		13	13	59	59		13	
May 18	0.3 u. insulin per kilo subcutaneously	Sugar	109							44*	
		Lactic acid	13							13	

* Convulsions.

† Pitressin or pituitrin was given 0.5 hour after insulin.

in method, it is possible that the initial bleedings were not done under basal conditions (our earlier work is subject to the same criticism) and that the effect of insulin was masked. The effect of insulin upon the blood lactic acid of three normal rabbits was included in the present study. The minimum convulsion-producing dose of insulin was determined for these rabbits. One was then given a subconvulsive dose, another the minimum convulsion-producing dose, and a third the minimum convul-

sion-producing dose plus 15 dog units of pitressin per kilo. The subconvulsion-producing dose of insulin, which lowered the blood sugar from 103 to 52, produced a rise in blood lactic acid from 10 to 34. In the case of the rabbit which was given a convulsion-producing dose of insulin, the bleeding was made prior to convulsion, the blood lactic acid having risen from 10 to 40. The blood sugar of the rabbit which had received pitressin only fell to 64, there being no significant rise in blood lactic acid. The values obtained by us at hypoglycemia are essentiallv the same as those

Rabbit 10

DATE	TREATMENT		BLOOD FINDINGS IN MG. PER 100 CC.							
			Initial	0.25 hour	0.5 hour	0.75 hour	1 hour	1.5 hours	2 hours	2.5 hours
Apr. 27	15 u. pituitrin per kilo subcutaneously	Sugar	106	136	103		95		111	
		Lactic acid	13		27		60		41	
May 2	15 u. pituitrin, 0.25 u. insulin per kilo subcutaneously	Sugar	96		83†	82	111	82	75	76
		Lactic acid			14	18	48		32	14
May 12	15 u. pitressin per kilo subcutaneously	Sugar	100	175		194	163		104	
		Lactic acid	13	31		50	30		15	
May 18	15 u. pitressin, 0.25 u. insulin per kilo subcutaneously	Sugar	107		†		85	93		71
		Lactic acid	13				17	40		15
May 29	0.25 u. insulin per kilo subcutaneously	Sugar	102				54			
		Lactic acid	13				13*			

* Convulsions.

† Pitressin or pituitrin was given 0.5 hour after insulin.

obtained by Cori (1925), which were 32 for lactic acid at a blood sugar level of 50, 45 for lactic acid at a blood sugar level of 47, 28 for lactic acid at a blood sugar level of 54. The difference in values occurs for the resting samples, in which ours were 10 or below and those of Cori, 20 to 35. In using the lactic acid determination as a criterion for adrenalin liberation in our operated rabbits, convulsion-producing doses of insulin were used, the bleedings being made just prior to convulsion. No rise above the initial value (5 to 14 mgm. per 100 cc.) occurred in any case (five experiments).

Totally adrenalectomized animals. Five totally adrenalectomized rabbits were given 20 units of pitressin per kilo subcutaneously, two the day following the last operation, the other three five to ten days following the last operation. In each case a decided rise in blood lactic acid occurred from a basal value near 10 to values of 70, 70, 46, 121, and 64, respectively. Two of these animals were about to expire a few hours after dosing and were sacrificed for analyses. The remaining three recovered temporarily, succumbing later to adrenal insufficiency. The longest survival period was twenty days. The animals were in a state of hypoglycemia (blood sugar values—50, 60) at the time they were sacrificed. The liver glycogen analyses were 20 and 35 mgm. per 100 grams respectively. The muscle glycogen values were 420 and 122 mgm. per 100 grams respectively.

TABLE 1

Carbohydrate reserves of medullary adrenalectomized rabbits following pitressin and pituitrin

	RABBIT 1	RABBIT 2	RABBIT 3	RABBIT 4	RABBIT 5	RABBIT 9	RABBIT 10
	Treatment						
	15 u. pitressin, 5 hour period	15 u. pitressin, 5 hour period	Control	Control	Control	15 u. pituitrin, 6 hour period	15 u. pituitrin, 6 hour period
Muscle sugar (total reducing power).	77	83	90	77	67*	68*	68*
Muscle sugar (fermentable)					37	30	32
Muscle glycogen	406	302	242	208	402	281	295
Liver glycogen	396	770	746	271	269		210

* Sampled under amytal anesthesia. The other animals were sacrificed by a blow on the head.

Carbohydrate reserves. In the final experiment, four of the medullary adrenalectomized rabbits were given 15 units of pitressin or pituitrin per kilo, three others serving as controls. The animals were sacrificed five and six hours after dosing. As explained in the introduction, four of the rabbits were stunned in order to secure glycogen samples. Three others were anesthetized with amytal. The results are given in table 1. Pituitrin or pitressin had no effect upon the muscle sugar at the fifth or sixth hour after dosing. The observation of Power and Clawson (1928) that muscle sugar values of animals under amytal anesthesia are lower than the muscle sugar values of animals sacrificed by stunning is confirmed.

The muscle and liver glycogen values show no significant changes when dosed and control values are compared, all the values falling within the range obtained for normal control rabbits, as determined by earlier work.

A larger series of animals was not studied because our earlier work had shown that in normal animals pharmaceutical doses of pitressin had no measurable effect upon the glycogen reserves, the present values obtained giving no indication to the contrary. Lawrence and McCance (1931) have recently shown that pituitrin is without effect upon the glycogen reserves of the fasting rat.

Histologic report. In sections of the adrenal fragments of rabbits 2, 4, 5, 9, and 10, only cortical tissue is present, and no medulla is seen. In the adrenal fragment of rabbit 3, in one small region only is there any sign of medullary tissue and here it is questionable and very small in amount. In sections of the adrenal fragment of rabbit 1, considerable amounts of medullary tissue are easily identified.

Discussion. In experiments with adrenalectomized animals, the question always arises as to the completeness of the operation. In our experiments, three criteria were applied. The response to insulin was rendered from ten to fifty times more sensitive after medullary adrenalectomy. At insulin hypoglycemia the animals failed to develop lactic acidemia, a defense mechanism which occurs in normal animals. Histological examination of the cortical fragments left *in situ* failed to show medullary tissue in five instances, with one doubtful and only one positive. Barring the animals which showed medullary tissue (one which did not convulse on 0.5 unit insulin), deductions can be made with reasonable assurance.

In all cases studied, whether in totally or medullary adrenalectomized animals or whether a hyper- or hypoglycemia was produced, both pitressin and pituitrin produced a lactic acidemia. *It is, therefore, definitely established that the production of lactic acidemia by pituitrin or pitressin is not a secondary reaction due to the liberation of adrenalin from the adrenals.*

The effect of pitressin and of pituitrin upon the blood sugar of the adrenalectomized animals is more difficult of interpretation, since in certain of them a definite hyperglycemia, in others a hypoglycemia, was produced. In some cases the experiments with totally adrenalectomized animals indicated a state bordering on hepatic deglycogenation, as shown by the production of hypoglycemia by a six hour fast for one animal, and the virtual depletion of liver glycogen for two other animals. The striking results in these experiments is *that pitressin brought about a hypoglycemia.* In the case of the medullary adrenalectomized animals, the production of hypoglycemia by pitressin and pituitrin in two animals must also be ascribed to hepatic deglycogenation. Since this effect only occurred a month after the operation (at which time the weight curve was stationary), and later disappeared, it is very likely that these animals suffered cortical deficiency at this period. At any rate, several months after the operation, when the animals showed a gain in weight and when, as the final glycogen values show, there was no evidence of deglycogenation, pituitrin and pitressin

decreased the effects of insulin hypoglycemia. In several of these experiments, the pituitrin and pitressin were given a half hour after the administration of the insulin, so that the result can not be ascribed to a decreased absorption of insulin from subcutaneous depots. *It is definitely established, however, that when pituitrin or pitressin produce a hyperglycemia they do not bring about the effect by the stimulation of adrenalin liberation.*

Clark (1928) found that the intravenous injection of pituitrin into decapitated, eviscerated cats increases the rate of disappearance of sugar from the blood. Pituitrin given after insulin failed to exert the inhibitory effect characteristic for normal animals. In cats under amytal anesthesia, in which the liver was cut out of the circulation, pituitrin did not produce hyperglycemia, nor did it diminish the rate of fall in blood sugar. Clark concluded that the source of glucose in pituitrin hyperglycemia is the liver glycogen. Imbrie (1929) came to the same conclusion. This author found in working with pancreatectomized animals that pituitrin produced a slight hyperglycemia when insulin was withheld forty-four hours, while a slight hypoglycemia was produced when insulin was withheld seventy hours, at which time adrenalin had no hyperglycemic effect.

Himwich, Fazikas, Haynes and Spiers (1930) found that under amytal anesthesia pitressin and pituitrin failed to produce a hyperlactic acidemia. The effects of diminishing the concentration of the plasma, the plasma fat and the blood sugar, were not so marked or even actually reversed under amytal. Barring the experiments of Clark performed under amytal anesthesia, Clark's experiments with eviscerated, decapitated animals, Imbrie's with pancreatectomized animals, and ours with adrenalectomized animals in a state of hepatic deglycogenation *would indicate that pitressin and pituitrin increase the removal of blood sugar by the peripheral tissues.*

It is essential to remember that the effects of pituitrin or pitressin upon carbohydrate metabolism are slight in comparison to the effects produced by insulin and adrenalin. In order to produce changes outside the limits of expected normal variation, pharmaceutical doses of pitressin or pituitrin must be given. Even so, no decided changes in the glycogen reserves can be demonstrated, and in the light of this observation it would appear almost paradoxical that an increased rate of utilization of blood sugar by the peripheral tissues would occur simultaneously with a decrease in metabolic rate (Himwich and Haynes, 1930). The purpose of the present paper was to establish whether or not pituitrin or pitressin functioned through the stimulation of adrenalin liberation. The answer is definitely negative. The production of hyperglycemia, hyperlactic acidemia, and the antagonistic action to insulin, were all observed in the adrenalectomized animals. Beyond this statement the present work can offer no elucidation.

SUMMARY

1. Pitressin and pituitrin produce consistently a hyperlactic acidemia in totally or medullary adrenalectomized rabbits. Simultaneously either a hypo- or hyperglycemia may be produced, depending upon the state of the hepatic glycogen reserves.

2. No significant changes in the peripheral glycogen reserves of medullary adrenalectomized rabbits could be demonstrated under the effects of pharmaceutical doses of pituitrin or pitressin.

3. Pitressin and pituitrin both diminish the hypoglycemic effect of insulin in medullary adrenalectomized animals, provided there is no hepatic deglycogenation.

BIBLIOGRAPHY

BEST, C. W. AND J. H. RIDOUT. 1925. Journ. Biol. Chem., lxiii, 197.
BISCHOFF, F. AND M. L. LONG. 1931. This Journal, xcvii, 215.
1930. Journ. Nutrition, iii, 201.
1929. Journ. Biol. Chem., lxxxiv, 629.
BRIGGS, A. P., I. KOECHIG, E. A. DOISY AND C. J. WEBER. 1923. Journ. Biol. Chem., lviii, 721.
BRITTON, S. W., E. M. K. GEILING AND H. O. CALVERY. 1928. This Journal, lxxxiv, 141.
CLARK, J. A. 1927–1928. Journ. Physiol., lxiv, 325.
CORI, C. F. 1925. Journ. Biol. Chem., lxiii, 253.
1931. Physiol. Rev., xi, 175.
CORI, C. F. AND G. T. CORI. 1929. Journ. Biol. Chem., lxxxiv, 683.
HIMWICH, H. E. AND J. FAZIKAS. 1930. Proc. Soc. Exp. Biol. Med., xxviii, 331.
HIMWICH, H. E. AND F. W. HAYNES. 1930. Proc. Soc. Exp. Biol. Med., xxvii, 815.
HIMWICH, H. E., F. W. HAYNES AND M. A. SPIERS. 1930. Proc. Soc. Exp. Biol. Med., xxviii, 332.
IMBRIE, C. G. 1929. Journ. Physiol., lxvii, 264.
LAWRENCE, R. D. AND R. A. MCCANCE. 1931. Biochem. Journ., xxv, 561.
POWER, M. H. AND T. A. CLAWSON. 1928. Journ. Biol. Chem., lxxviii, lvi.

THE EFFECTS OF THYROID FEEDING ON THE HEART RATE IN NORMAL DOGS AND IN DOGS WITH COMPLETELY DENERVATED HEARTS

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This work has been conducted to investigate the rôle of the extrinsic cardiac nerves in the tachycardia resulting from thyroid feeding in dogs. The literature on the problem is not extensive and may be divided into two main classes.

I. Acute experiments: a, experiments dealing with the effects of thyroxine on the perfused frog heart; b, experiments on the anesthetized dog studying the effect of the "thyroid hormone" on the heart rate.

II. Chronic experiments: The effect of feeding thyroid substances to normal animals and to animals with denervated hearts.

I. Experiments by Kalnis (1) on the perfused frog heart, using different concentrations of thyroxine, demonstrated that the effect of adrenaline could not be increased by previous perfusion by thyroxine. He also noted a slightly slower heart rate and an increased amplitude of beat under thyroxine perfusion. He concluded that there was no evidence to show stimulation of the sympathetic system by thyroxine.

Waldenstrom (2) studied the effect of thyroxine on vagus action in the perfused frog heart. He reports that in no case did the addition of thyroxine modify the vagus inhibition.

Cannon (3) using the denervated heart of the anesthetized cat reported an increase in heart rate following massage of the thyroid gland, and also on stimulation of the cervical sympathetic. This effect was noticed after a delay of from 30 to 60 minutes. The reason for this effect is not clear, as it has not been conclusively shown that there is a definite increase in secretion of the thyroid gland during such procedures. Carlson and Hektoen (4) have shown that there is no demonstrable increase in the production of thyroglobulin, as tested by the precipitin reaction either on stimulation of the sympathetic fibers to the gland or on massage. Van Dyke (5) found no change in the iodine content of the gland on stimulation of the cervical sympathetic. Smith and Couta-Silva (6) found no increase in basal metabolism of dogs after massage of the thyroid gland. Kunde (7) has shown that there is no observable effect on the heart rate for at least

ten hours following the injection of thyroxine or after feeding dried thyroid gland. This was true also in the thyroidectomized dog. II. Enderlen and Bohnenkamp (8) have conducted a series of experiments on thyroid feeding in normal dogs and in dogs with denervated hearts. Dogs were subjected to the Friedenthal operation in which the vagi are sectioned in the chest and the upper thoracic sympathetic chains are removed. Five grams of Merck's thyroid were fed daily to a group of normal and operated animals. At the beginning of the experiment the dogs weighed from 15 to 18 kilograms, thus making the daily dose of thyroid around 0.3 gram per kilogram of body weight. The feeding covered a period of from seven to eight weeks, during which time the control animals lost weight, showed auricular flutter and died, while the operated animals showed no heart changes and gained from one to three kilograms. They concluded that thyroid substances act on the heart only by way of the cardiac nerves. The early death of the normal dogs fed this dose of thyroid as well as the gain in weight in the heart denervated dogs on the same dose is contrary to the experience of this laboratory.

The following experiments have been performed in an effort to study the problem in a more extensive way. It was desirable to prepare dogs with completely denervated hearts and at the time when this phase of the work was started, it was felt that sectioning both vagi and removing the stellate ganglia would give a heart rate which would not vary except when acted upon by humoral means. Six dogs were prepared. The experimental period extended from October 20, 1929 to August 25, 1930.

Heart rates. The normal heart rate with normal variations was secured on each dog prior to operation. The animal was taught to rest quietly on a table. The pulse rate was measured by either counting the beat in the femoral artery or by means of a tambour on the apex beat. These methods gave the same results and it was felt in the later work that it was unnecessary to continue with the graphic records as they took more time than their value warranted. The rate was measured when the animal was quiet and relaxed after an initial one-half hour rest. The rate was also taken at every disturbance during the half-hour following the rest period.

Operations. Each animal was subjected to four operations: 1, right stellate ganglion; 2, left stellate ganglion; 3, right vagus; 4, left vagus. The stellate ganglion was removed by entering through the midline on the dorsal side, cutting through the rhomboids and laying back the scapula. Muscle tissue between the first and second ribs was removed and the ganglion was brought into view. It was cut free from all its connections and removed. Usually no pneumothorax occurred. If it did, all speed was made in removing the ganglion and closing the wound. The muscles were sutured and the skin wound closed. The recovery was usually good. It was found not advisable to remove both ganglia at the same time as the shock was too severe.

The original intention to cut both vagi below the origin of the recurrent laryngeal nerves from a midline neck incision was not carried out as it was suggested that all fibers might not be reached. For this reason it was necessary to enter both sides of the chest and section these nerves where all fibers could be seen. This was done under artificial respiration. Tracheotomy was performed as it was found that forcing a large enough tube through the larynx to get positive pressure did more damage than cannulating the trachea. The chest was entered between the third and fourth ribs and the vagus was brought into view. A section of nerve about one and one-half inches long was removed below the origin of the recurrent laryngeal nerves. The incision was closed while the lungs were filled.

RESULTS. Four out of the six dogs survived the four operations in good health. In dog I, ten days following the section of the second vagus, chronic vomiting began. The animal did not improve and was sacrificed twenty-seven days after the operation. The rate in this dog is included in the tables. The figures represent heart rates taken when the animal was normal and in good condition. The same set of effects (chronic vomiting) was seen in dog V. This dog was sacrificed seven days after the second vagus was cut. The animal was normal for three days following this operation, and so the heart rate is included for this period.

Criteria of denervation. Immediately after removal of the stellate ganglion the nictitating membrane spread over the eye. The membrane withdrew after some days except in dog I where it persisted over the right eye from October 25, 1929 to July 1, 1930. If no cardiac slowing was obtained on electrical stimulation of the vagus at the level of the inferior cervical ganglion, it was inferred that all vagus fibers to the heart had been sectioned.

At the end of the experiment these animals were injected with atropine (3 mgm.). All showed cardiac acceleration. It was thought that vagus regeneration had taken place, but on electrical stimulation of the vagus under anesthesia, only one vagus in one dog (dog III) was found to have functionally regenerated and on autopsy this was confirmed macroscopically. The other dogs showed no evidence of vagus or sympathetic regeneration.

The acceleration of the denervated heart after injection of atropine has been observed by several workers. Enderlen and Bohnenkamp (8) described acceleratory effects which they attributed to the "intracellular" action of the drug. Dresbach and Waddell (9) obtained acceleration in the heart rate of the cat in which the extrinsic cardiac nerves had been cut. Acceleration of the heart rate in the animal with both vagi cut following the injection of atropine has been described in the dog by Rogers (10) and in the cat by Cannon (11). Carlson (12) described a stimulation of the heart ganglion in limulus upon the addition of atropine to the ganglion.

In table 1 are represented the changes in heart rate following the different operations. Basal heart rate represents that heart rate obtained after one-half hour rest, during which time there had been no disturbances. "Maximal variations" represent the greatest variations occurring during the following one-half hour rest period due to such factors as wagging the tail, moving the head, etc., etc. Each figure represents an average of twenty counts on successive days unless otherwise indicated (*).

It was felt that since the heart still accelerated on slight movements, all the extrinsic cardiac fibers had not been removed. Stellatectomy and

TABLE 1

Average heart rates before and following the different operations

DOG	PERIOD OF EXPERIMENT	NORMAL		AFTER EXTIRPATION OF STELLATE GANGLIA		AFTER SECTION OF CARDIAC VAGI	
		Basal heart rate	Variations	Basal heart rate	Variations	Basal heart rate	Variations
I	October 25, 1929 July 1, 1930	73	+23	70	+24	70	+23
II	November 14, 1929 August 21, 1930	92	+34	91	+30	90	+29
III	November 20, 1929 August 21, 1930	84	+28	80	+26	80	+27
IV	November 25, 1929 August 25, 1930	88	+14	86	+15	85	+14
V	November 30, 1929 February 15, 1930	70	+20	71	+20	68	+20
VI	December 2, 1929 August 24, 1930	74	+18	72	+14	73	+15

vagotomy had been complete in these animals. Cannon (13) has described in the cat sympathetic fibers which leave the thoracic chain as low as the fifth or sixth ganglion and pass directly to the heart. Dresbach and Waddell (9) confirm these findings. Perman (14) has demonstrated similar findings in the calf. The author made careful dissections in ten dogs and in six very fine fibers were seen leaving the sympathetic chain as far down as the sixth thoracic ganglion. These could not in all cases be traced the entire way to the heart, but their general direction indicated the heart as a possible destination. In three of the dogs the fibers were traced to the aorta.

It was thought desirable to prepare a series of animals in which the entire

thoracic sympathetic chains were removed and the vagi sectioned. The splanchnics were cut in order to denervate the adrenals, thus lessening any heart effects due to this factor. The abdominal sympathetic chains were removed for the same reason.

Operations. Three operations were required. The work was done under ether anesthesia, using a modified Meltzer tracheal insufflation apparatus (15). Compressed air was led through an ordinary ether bottle and by means of a small rubber catheter to the bifurcation of the trachea. To obtain positive pressure a Kunde mask was placed over the snout. In the mask was an inlet carrying the catheter and an outlet carrying the expired air down a tube into a water bottle. By raising or lowering the tube in the water the lungs could be inflated or deflated as desired. The lungs were partially distended throughout the operation. Respiratory exchanges seemed normal in all the dogs operated. The administration of ether was smooth and easily regulated.

The normal basal heart rate was obtained and then the animal was subjected to the three operations. Four dogs survived these procedures in good health. Two dogs of approximately the same weight and age were chosen as controls for the adult dogs and one litter mate for the puppy studied. Small dogs were chosen as it was found that the thoracic sympathetic chain could thus be removed through one incision.

Operation I. The right thoracic sympathetic was removed from the stellate ganglion to the diaphragm. The right vagus was sectioned below the origin of the recurrent laryngeal nerve. Any fibers running from the recurrent to the heart were sectioned. The right splanchnic was cut. The vessels above the heart were stripped to remove as much of the nerve net as possible.

Operation II. The left thoracic sympathetic was removed from the stellate ganglion to the diaphragm. The left vagus was dissected free from the connective tissue surrounding it and all thoracic branches were cut, thus leaving the vagus to the gut intact. The left recurrent laryngeal is cut, but with the right intact the animal suffers no ill effects. The vessels above the heart were stripped.

Operation III. The abdominal sympathetic chains were removed and the splanchnics again sectioned. These operations were carried out as described with the following exceptions. In dog V the left splanchnics could not be reached in the chest and since it is not possible to reach all fibers in the abdomen the left adrenal was removed. In dog VI, the entire left vagus was cut at the level of the aorta.

Criteria of the effectiveness of heart denervation were: 1. Nictitating membrane drawn over the eye immediately after operation. 2. No cardiac slowing when the vagi in the neck were electrically stimulated. 3. A steady heart rate after one-half hour rest which did not vary when the

animal moved slightly. In control animals, the slightest movement always gave a greatly accelerated heart rate.

Heart rates. The heart rate was taken after one half-hour of rest. After again taking the rate at forty minutes, the animal was disturbed in some way and the rate again taken.

Basal metabolic tests. On two dogs, one operated (dog V) and one control (dog I) basal metabolism tests were run. This was done in order to measure the effectiveness of the thyroid dosage. These tests were run eighteen hours after feeding, and after an initial half-hour rest.

Thyroid dosage. After a control period had been run on each dog, thyroid feeding was begun, giving each dog 0.6 gram per kilo per day. Armour's thyroid was used. The thyroid mixed with milk was fed before the regular food was given. All dogs seemed fond of the mixture. The cages were watched to see whether any was lost in vomiting. This did not occur.

TABLE 2

DOG NUMBER	AVERAGE BASAL HEART RATE DURING TWO WEEK CONTROL PERIOD	EFFECTS OF SLIGHT MOVEMENTS
Control dogs		
I	74	+31
II	77	+18
IX	92	+33
Operated dogs		
V	57	+2
VI	68	+1
VII	66	+2
X	82	+1

Diet. The dogs were kept in a constant diet throughout the experimental period. Enough food was given to maintain a constant body weight under normal conditions. During thyroid administration the same amount of food was fed. In the case of the two puppies, the normal diet was fed ad libitum. The diet consisted of:

Ground meat.....50 per cent
Bread.....25 per cent
Milk.....25 per cent
Bone meal.....

Electrocardiograms. On all dogs studied, electrocardiograms were run weekly before and during thyroid feeding. In the tracings the following measurements were made: 1, duration of P wave; 2, direction and height of P wave; 3, P-R interval; 4, direction, normality and height of R wave; 5, duration of QRS; 6, direction and height of T wave; 7, QRS-T (systole).

Results. 1. In the animals subjected to the second series of experiments, immediate acceleration in the heart rate following slight movements was practically abolished (see table 2).

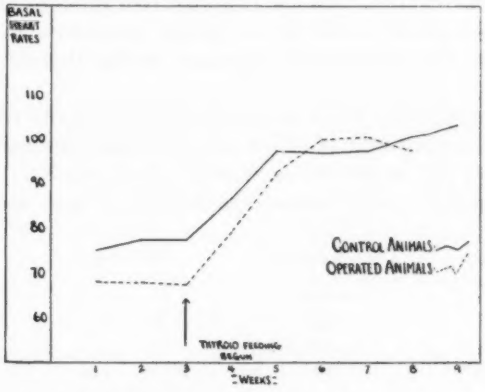

Chart 1. Average weekly heart rates in the control and operated groups before and during thyroid feeding.

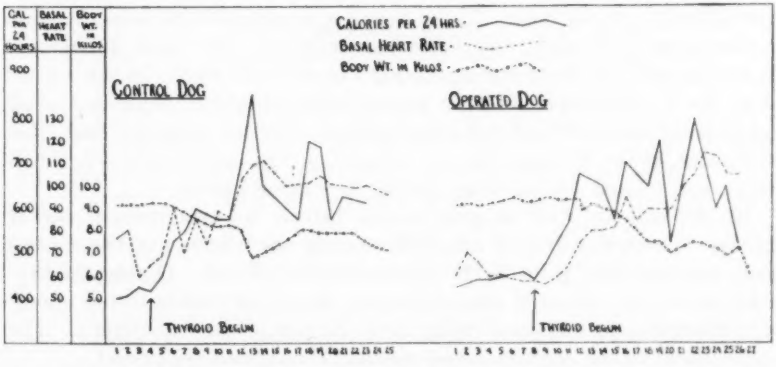

Chart 2. Variation in basal heart rate, basal metabolic rate and body weight before and during thyroid feeding. Small figures at bottom of chart indicated three day intervals.

2. Effects of more extensive movements and exercise, such as running, etc., were not studied. It was noticed that these hearts accelerated after the ingestion of food.

3. The basal heart rate was lower (5–10 beats) following heart denervation.

4. Dog VI was the only dog in the series which showed erection of the hairs in fighting. This disappeared after sympathectomy.

5. In all the operated dogs and in two of the controls, the vagi were stimulated aseptically before the experimental period was begun. No cardiac slowing was observed in the operated dogs. In the control animals complete cardiac inhibition was seen lasting from three to five seconds.

6. In all the animals of the second group, operated and controls, the basal heart rate was considerably increased during thyroid feeding (see chart 1).

7. In those animals on which metabolism tests were run the basal heart rate was found to vary with the basal metabolic rate (see chart 2).

8. All animals lost weight during thyroid administration.

The following gives the maximum loss of weight in each dog:

DOG NUMBER	CONTROL DOGS	DOG NUMBER	OPERATED DOGS
	per cent		*per cent*
I	25	V	33
II	45	VI	30
IX (puppy)	13	VII	32
		X (puppy)	7

9. There were no marked differences in the electrocardiograms of the normal and operated dogs. Table 3 gives a summary of the measurements made before and during thyroid administration. The main differences, it will be seen, are those accompanying a faster heart rate. In the control dogs the T wave became larger during thyroid feeding. This is a usual accompaniment of hyperthyroidism in man. In the operated dogs, this difference in the T wave was not noticeable. In dogs V and VI, the T wave was 1.5 and 1.0 less than during the control period.

10. All animals were in good health before the experimental period began. There was little, if any, difference in the behavior of the control and operated dogs, prior to the thyroid feeding period. During the thyroid period, the operated animals became weak and unable to run about. The control dogs remained lively until the end of the experiment. The appetites in all the dogs remained excellent throughout the period.

11. Three of the operated dogs died after from four to seven weeks on thyroid feeding.

Chronic vomiting in dog VI (beginning two weeks after thyroid administration was started) led to pneumonia and death.

Dog X died of pulmonary embolism.

Dog V became weak and died. No pathology was found.

Only one control dog died during the experimental period. The death was similar to that observed in dog V. Both dogs were extremely emaciated. The other dogs, I, IX and VII were killed by ether after taking electro-cardiograms before and during vagus stimulation. The control dogs

showed typical cardiac inhibition. A slight effect was seen on stimulation of one vagus in the operated dog. Autopsy in all of the operated dogs revealed no regeneration of either sympathetic or vagus fibers, except in dog VII, where some fibers leaving the left vagus were seen coursing toward the heart.

TABLE 3

Showing the averages of the measurements made on the electrocardiographic tracings before and during thyroid administration

DOG NUMBER	DURATION OF P WAVE	HEIGHT OF P WAVE	P°-RINT.	HEIGHT OF R WAVE	DURATION OF QRS	HEIGHT OF T WAVE	QRS-T	ABNORMALITIES
	seconds	*mm.*	*seconds*	*mm.*	*seconds*	*mm.*	*seconds*	
I. Control period....	0.04	3.0	0.15	14.0	0.045	3.0	0.22	None
Thyroid.....	0.14	3.9	0.12	14.0	0.041	7.1	0.24	Few extra P waves
II. Control period...	0.032	2.1	0.106	14.0	0.044	2.5	0.24	None
Thyroid.....	0.032	2.6	0.98	16.7	0.041	5.0	0.26	Few extra P waves
V.* Control period...	0.052	2.8	0.130	21.3	0.045	5.1	0.23	None
Thyroid.....	0.040	3.2	0.108	19.4	0.045	3.6	0.25	None
VI.* Control period...	0.052	3.0	0.12	11.5	0.046	3.5	0.25	None
Thyroid.....	0.040	3.3	0.114	10.3	0.042	2.5	0.253	None
VII.* Control period..	0.052	2.3	0.100	14.5	0.040	4.0	0.255	None
Thyroid.....	0.040	4.7	0.104	9.6	0.046	5.6	0.26	None
IX. Control period..	0.044	2.93	0.114	15.1	0.058	4.6	0.233	None
Thyroid.....	0.038	2.83	0.99	19.1	0.040	6.6	0.249	None
X.* Control period..	0.056	2.45	0.12	16.5	0.040	3.25	0.21	Few extra P waves
Thyroid.....	0.040	3.0	0.112	11.0	0.038	4.0	0.23	Few extra P waves

* = operated dogs.

DISCUSSION. The work on the denervation of the dog heart confirms Cannon's findings (13) in the cat. The heart rate in dogs after complete sympathectomy and vagotomy is different from that in dogs in which vagotomy and removal of the stellate ganglia had been performed in that quick acceleration due to slight movements was not seen in the dogs with completely denervated hearts.

There was no marked difference in the response of the heart in the operated and control animals to thyroid administration. This work therefore

clarifies the problem of the mechanism of the tachycardia of hyperthyroidism to the extent that the thyroid action is not primarily via the extrinsic cardia nerves.

The findings of Enderlen and Bohnenkamp (8) of auricular flutter and death in the control animals, and no effect on the operated animals, are not confirmed. No true auricular flutter was seen in any of these animals. It might be argued that the experiments did not extend over a long enough period. Two dogs were fed the described dose for over eight weeks. The rest of the series varied from four to seven weeks. This period is comparable to that described by the above authors. The dosage used was twice that used by these workers.

CONCLUSIONS

1. The heart rate in the dog with complete cardiac denervation does not change in response to slight movements. This is not the case when vagotomy and removal of the stellate ganglia are performed.

2. The tachycardia of chronic experimental hyperthyroidism is practically identical in dogs with complete heart denervation and in dogs with all the cardiac nerves intact.

3. The rate of loss of body weight and temperature elevation on comparable doses of thyroid hormone is the same in the normal and in the heart denervated dogs, but the latter animals are more incapacitated (asthenia) by the hyperthyroidism.

The author wishes to express her appreciation of Mr. Edward Wallace's assistance in the electrocardiographic work and her thanks for the advice of Dr. A. J. Carlson throughout the course of the experiments.

BIBLIOGRAPHY

(1) KALNIS: Compt. Rend. Soc. de Biol., 1928, xcviii, 802.
(2) WALDENSTROM: Compt. Rend. Soc. de Biol., 1928, xcix, 1681.
(3) CANNON: This Journal, 1922, lx, 476.
(4) CARLSON AND HEKTOEN: This Journal, 1927, lxxxi, 487, 661.
(5) VAN DYKE: This Journal, 1921, lxi, 168.
(6) SMITH AND COUTA-SILVA: This Journal, 1928, lxxxviii, 183.
(7) KUNDE: This Journal, 1927, lxxxii, 195.
(8) ENDERLEN AND BOHNENKAMP: Deutsch. Zeitschr. f. Chir., 1927, cc, 129.
(9) DRESBACH AND WADDELL: Journ. Pharm. Exper. Therap., 1926, xxvii, 9.
(10) ROGERS: This Journal, 1920, liii, 15.
(11) CANNON: This Journal, 1930, xciv, 201.
(12) CARLSON: This Journal, 1906, xvii, 177.
(13) CANNON: This Journal, 1926, lxxvi, 326
(14) PERMAN: Zeitschr. f. anat. u. Entwicklung., 1924, lxxi, 382.
(15) LIVINGSTONE AND HRDLICKA: Journ. Lab. Clin. Med., 1930, xvi, 74.

MENSTRUATION IN PITHECUS (MACACUS) RHESUS FOLLOWING BILATERAL AND UNILATERAL OVARIECTOMY PERFORMED EARLY IN THE CYCLE

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It has been shown that the removal of the ovaries in the last half of the cycle is followed by early menstruation. Since ovulation takes place in the middle of the cycle (Corner, 1923), ovariectomy performed at this time or later may remove glands which contain the maturing follicle or corpus luteum. The following experiments were planned to determine whether or not menstruation could be induced by operation early in the cycle; and if so, to study the histological structure of the ovaries removed and the character of the resulting endometrial bleeding.

Allen (1927, 1928) has reported post-operative bleeding in seven monkeys following bilateral ovariectomies performed from the tenth to the twentieth day of the cycle, and in other cases after aspiration of fluid from a large follicle. Hartman[1] observed menstruation in four monkeys following the removal of one ovary on the fourteenth to seventeenth days of the cycle.[2]

The present study was undertaken to find the results of ovariectomy during the first days of the cycle before the large follicles incident to ovulation appeared. The operations took place in June and July, months which lie within the non-breeding season of the macaque (Heape, 1897; Van Hewerden, 1906; Zuckerman, 1930) when as has been found by

[1] Dr. C. G. Hartman has kindly sent us a chapter on laparotomies and their effects in the macaque from a monograph ready for publication.

[2] In man, removal of a follicle or corpus luteum by excision or cautery was found by Fraenkel (1903) to delay menstruation; by Seitz and Wintz (1919) and Ostreil (1924), when the operation took place in the first half of the interval, to be followed by menstruation within three and a half weeks or, if taking place in the last half of the interval, bleeding followed on the second day. Halban and Koehler (1914), removing corpora lutea and in two cases removing both ovaries, found menstruation in two to four days and lasting three to eight. Pratt (1927) gave the results of thirty cases in which the corpus luteum was removed, and in all but two there was an induced flow within forty-eight hours. Two double ovariectomies in the first half of the cycle produced menstruation, while one on the sixth day of the cycle did not.

Hartman (1931) ovulation is usually in abeyance. It is to be expected, then, that ovaries removed during the non-breeding season and before the tenth day of the menstrual cycle will not contain the two special structures (the maturing follicle and the young corpus luteum) which most often have been associated with the immediate control of the menstrual cycle.

Nine rhesus monkeys in a good state of nutrition, with an average weight of 4,454 grams, were used. All of these animals had menstruated and four were of proved fertility. Each animal occupied a separate cage and a sand-scrubbed board was so placed that it was necessary for the monkey to climb upon it before the activities of the other animals in the room could be observed. Consequently, the monkeys spent most of the time on these white benches, which were changed each day. For this particular study, the following definition of menstruation has been established: the appearance of fresh or dried blood upon the external vulva of the animal or upon the white bench of the cage. The records, then, are not as sensitive as those given by the lavage method (Hartman, 1930). Individual cells or small amounts of blood withdrawn from the vagina by the lavage might have shown day 1 to have come twenty-four hours earlier than our records and probably would have indicated red blood cells lingering a day longer in the vagina. The data presented here seem to fulfill the ordinary requirements for the establishment of the fact of menstruation and its duration. They will have the added advantage of ease of correlation with clinical data. The first day on which bleeding was observed has been called day 1 of the new cycle.

The operation was performed under surgical anesthesia. The midline abdominal incision began at a point half way between the umbilicus and the pubis and extended well down to the symphysis. The ovaries were brought into view and described before excision. The uterus was left untouched, in order to avoid any possible bleeding as the result of trauma.

PROTOCOLS. Series A: Bilateral ovariectomy. No. 11. Received May 1, 1931. Bilateral ovariectomy on June 8, 1931 (first day of cycle).

Menstrual history. On arrival the sexual skin of the animal showed very little color; no fluctuations were noted in this perineal coloring and bleeding began on June 8.

Operation. The animal weighed 4,825 grams. Observation of the ovaries before their removal showed the surface of each to be intact and there was no external indication of the presence of a large follicle or corpus luteum. Menstruation continued without interruption the next four days. This bleeding, which was the animal's last, had been initiated before the operation and was not of unusual length. *No subsequent menstruation was observed associated with double ovariectomy performed on the first day of the cycle.*

Ovaries. The left gland weighed 0.199 gram and contained three follicles 1 to 1.4 mm. in diameter, no recent corpora.

The right gland weighed 0.200 gram and contained one atretic follicle 3.6 × 3.5 mm.; follicular wall thin; no mitosis; degenerating cumulus; four other follicles under 1 mm. in diameter; no recent corpora.

No. 9. Received April 10, 1931. Bilateral ovariectomy on May 27, 1931 (fourth day of cycle). Autopsy June 3, 1931.

Menstrual history. On arrival, the sexual skin was not bright in color but after five days increased in intensity; it remained at maximum from May first to eighth, after which it receded. The first observed menses occurred on May 24 and continued for 4 days.

Operation. This animal weighed 5,050 grams and was still bleeding. Although ovariectomy was performed on the fourth day of the cycle, actually only 72 hours passed after the beginning of menstruation and before operation. The left ovary showed three small transparent areas. The right ovary was somewhat smaller. *External bleeding from the vagina appeared June 2, an interval of five days after double ovariectomy and only 9 days after the first day of the preceding menstruation.*

Ovaries: Left (fig. 1): weight 0.365 gram. Many follicles less than 1 mm. in diameter. One retrogressing corpus luteum 3.1 × 2 mm.

Right (fig. 2): weight 0.319 gram. One retrogressing corpus measuring 2.3 × 1.5 mm.

Uterus (fig. 3): The animal was killed on the second day of the operative menses. On palpation the uterus felt soft and spongy. The genital tract intact was dropped into Bouin's fluid. After ten minutes' fixation it was cut longitudinally. The lumen was found filled with free and clotted blood. Sections show a low endometrium, straight glands, hematomata and destruction of the superficial portions of the glands and stroma.

No. 2. Received February 26, 1931. Bilateral ovariectomy May 15 (fifth day of cycle). Autopsy, May 26, 1931.

Menstrual history. A nursing young female had been weaned on February 23, when the animal was purchased. On arrival in the laboratory, the sexual skin was brightly colored. The color diminished rapidly two days prior to the appearance of the menses, which occurred on April 9. Bleeding lasted five days and the next menstruation, which was of 3 days' duration, began on May 11, after a cycle of 32 days.

Operation. The animal weighed 6,300 grams. When the ovaries were exposed, their capsules appeared smooth, without rupture points or indication of a large superficial follicle. The ovaries were removed, and *after an interval of six days there was a menstruation lasting 5 days, which occurred only eleven days after the first day of the preceding menstruation.*

Ovaries. The left ovary (fig. 4) contained a follicle measuring 2 × 3.4 mm., lying deep in the gland and showing many mitotic figures. There are several follicles under a millimeter and one follicle measuring 1.2 mm.

The right ovary contained a follicle 2.7 × 2 mm. and showing mitotic figures and a retrogressing corpus luteum measuring 3.2 × 2.4 mm.

Uterus (fig. 5). The animal was killed on the fifth day of bleeding and a small amount of dried and fresh blood was present on the vulva. However, when at autopsy the genital tract was removed and the uterus opened by an anterior longitudinal section, the cavity did not appear to contain blood. Histological examination showed a low endometrium, deep destruction of straight glands and stroma and extravasation of blood.

No. 1. Received February 18, 1931. Bilateral ovariectomy, April 24, 1931 (ninth day of cycle).

Menstrual history. The animal dealer said she had given birth to a normal, viable, female baby in September 1930. Milk was observed pressed from the left nipple when she manipulated it. When received, the color of the sexual skin was not intense and a menstruation of three days began February 21. Increase in coloration and perineal swelling began March 26 and lasted through April 9. Next menses began April 16 and lasted through April 20, after a cycle of 54 days.

Operation. Weight of animal was 4,500 grams. The surfaces of the ovaries were smooth and there was no indication of a large or superficial follicle or of a rupture point. *External bleeding from the vagina was evident May 1, an interval of six days after the operation and 15 days after the beginning day of the preceding cycle.*

Ovaries. The left gland contained small follicles, the largest in cross section measuring 2.2 × 1.5 mm. There were no recent corpora. The right gland contained six follicles less than 1 mm. in diameter and one large follicle measured 1.1 × 1.2 mm. in diameter. There were no recent corpora.

No. 4. Received March 12, 1931. Bilateral ovariectomy, April 24, 1931 (thirteenth day of cycle).

Menstrual history. This animal was received with a nursling, a young female which was weaned on April 10. The following days the breasts of the mother were much distended. A menstruation beginning April 12 lasted five days. No marked fluctuation of perineal color has been evident in this animal.

Operation. Animal weighed 5,175 grams. Examination of the ovaries before excision showed the capsule of the left gland to be smooth and without any follicular distention. The right ovary was larger than the left, and on its surface was a small torn area. *There was external bleeding from the vagina on May 1, an interval of six days after double ovariectomy and nineteen days after the first day of the preceding menstruation.*

Ovaries. The left ovary showed no follicles over 1 mm. in diameter and a retrogressing corpus luteum 1.7 × 1.2 mm. The right gland contained a large collapsed ruptured follicle, the rupture point of which must have been the scar on the surface noticed at operation. The egg lay within the cumulus attached to the wall of the follicle and since many cells were in the state of mitosis, it appears that this follicle ruptured prematurely. The wall of the follicle was thrown into many folds, but measured only 3 mm. in longest diameter of the section. No recent corpora present.

Series B: Unilateral ovariectomy. No. 8. Received April 10, 1931. Unilateral ovariectomy. June 8, 1931 (first day of cycle).

Menstrual history. When received, there was little color of the sexual skin and the animal was bleeding. This menstruation continued three more days. There was no increase in perineal coloration, although two pouches over the pubis became distended during the last week in May. The second observed menses began June 8. The animal appeared to be young. Its weight was less than the weights of others in the series.

Operation. The animal weighed 3,125 grams. The right ovary was smaller than the left. The left ovary was removed, after both had been examined and found to be without surface evidence of large follicles or corpora. *No menstruation followed which could be associated with the unilateral ovariectomy in this animal.* The next menses occurred on July 10 to 17, after a cycle of 32 days.

Ovary. Weighed 0.116 gram. Two follicles less than 1 mm. in diameter; no recent corpora.

No. 12. Received May 1, 1931. Unilateral ovariectomy, June 4, 1931 (fourth day of cycle).

Menstrual history. Moderate change in perineal color during 31 days preceding menstruation. Bleeding began June 1 and lasted 4 days.

Operation. The animal weighed 3,500 grams. When the abdomen was opened, both ovaries were brought into view. They were of about the same size. Neither gland appeared to contain a large follicle, and there was no indication that either gland contained a corpus luteum. The left ovary was removed. *External bleeding from the vagina appeared on the fourth day after unilateral ovariectomy and only seven days after the first day of the preceding menstruation.* This operative cycle of seven

days was followed by one 24 days long. The next menstruation occurred on July 2 and 3.

Ovary. The left ovary (fig. 6) weighed 0.105 gram. It contained one follicle 1.3 × 1.2 mm.; one 0.7 × 1.5 mm.; three follicles under 1 mm. in diameter; one retrogressing corpus 3.1 × 1.2 mm. in diameter.

No. 20. Received June 17, 1931. Unilateral ovariectomy, June 25, 1931 (fourth day of cycle).

Menstrual history: The sexual skin was shrunken and very light in color when received in the laboratory. Menstruation began on June 22.

Operation: Weight, 3,650 grams. The animal was still menstruating. The left ovary appeared to contain an opaque structure thought to be a retrogressing corpus luteum; so the right ovary, which gave no surface indication of containing a large follicle or corpus luteum was removed. The two ovaries were of about the same size. *An early menstruation did not follow this operation.* On July 22, the next menses appeared, after a cycle of 30 days.

TABLE 1

ANIMAL NUMBER	DAY OF CYCLE ON WHICH OPERATION WAS PERFORMED	DAYS ELAPSING BEFORE APPEARANCE OF INDUCED MENSES	LENGTH OF OPERATIVE CYCLE
Series A: Bilateral ovariectomy			
			days
11	1		
9	4	5	9
2	5	6	11
1	9	6	15
4	13	6	19
Series B: Unilateral ovariectomy			
8	1		
12	4	3	7
20	4		

Ovary weighed 0.156 gram. One follicle 1.2 × 1.2 mm., three follicles about 0.7 mm. in diameter. No recent corpus luteum.

Control operation. No. 3. Received February 26, 1931. Control laparotomy June 4, 1931 (seventh day of cycle).

Menstrual history: Nursing young weaned February 23. On March 13 genital swelling was marked, which receded about the 17th. First observed menses on March 22, lasted four days. Second menses on April 18, lasted three days, giving a 27-day cycle. Third menses May 29, lasting four days, gave a 41-day cycle.

Operation: The animal weighed 3,965 grams. Laparotomy was performed exactly as in other operations. Both ovaries were brought into view for observation and the uterus moved only through tension on the ovarian ligaments. There was no indication that either gland contained corpora lutea or large follicles. No menstruation followed which could be associated with the control operation in this animal.

Table 1 summarizes the time of operation, the interval before induced menstruation and the length of the resulting shortened cycle for the five

bilateral ovariectomies comprising series A and the three unilateral operations of series B. The ninth animal was reserved for a control laparotomy (protocol, animal 3). No induced menstruation followed either bilateral or unilateral ovariectomy when performed on the first day of the cycle. Removal of both ovaries on the fourth, fifth, ninth and thirteenth days of the cycle was followed by menstruation on the sixth or seventh day. Excision of a single ovary in no. 12 was followed by menstruation on the fourth day, but in no. 20 the removal of one ovary on the fourth day did not induce menstruation. Table 2 compares the length of the normal with that of the operative menses for the individual animal.

TABLE 2

Lengths of normal and operative menses in days

ANIMAL NUMBER	NORMAL	NORMAL	OPERATIVE
9		4	2†
2	5	3	5†
1	3	5	2
4		5	5
12		4	2

† Autopsied.

Histological study of the ovaries from the seven animals ovariectomized on or before the ninth day of the cycle showed the glands to contain no active follicles of maturation size and no recent corpora lutea. The right ovary of animal 4 operated upon on the thirteenth day of the cycle contained a large and actively growing (a great number of mitoses present) follicle. In monkey 9 the bleeding was induced by removal of ovaries containing follicles less than a millimeter in diameter. This is in agreement with Hartman, who found that unilateral excision late in the cycle of ovaries which contained no mature follicle or corpus luteum resulted in menstruation.

The uteri from animals 9 and 2 (figs. 3 and 5) show the menstruating

Plate I. Photographs from series stained with iron hematoxylin.

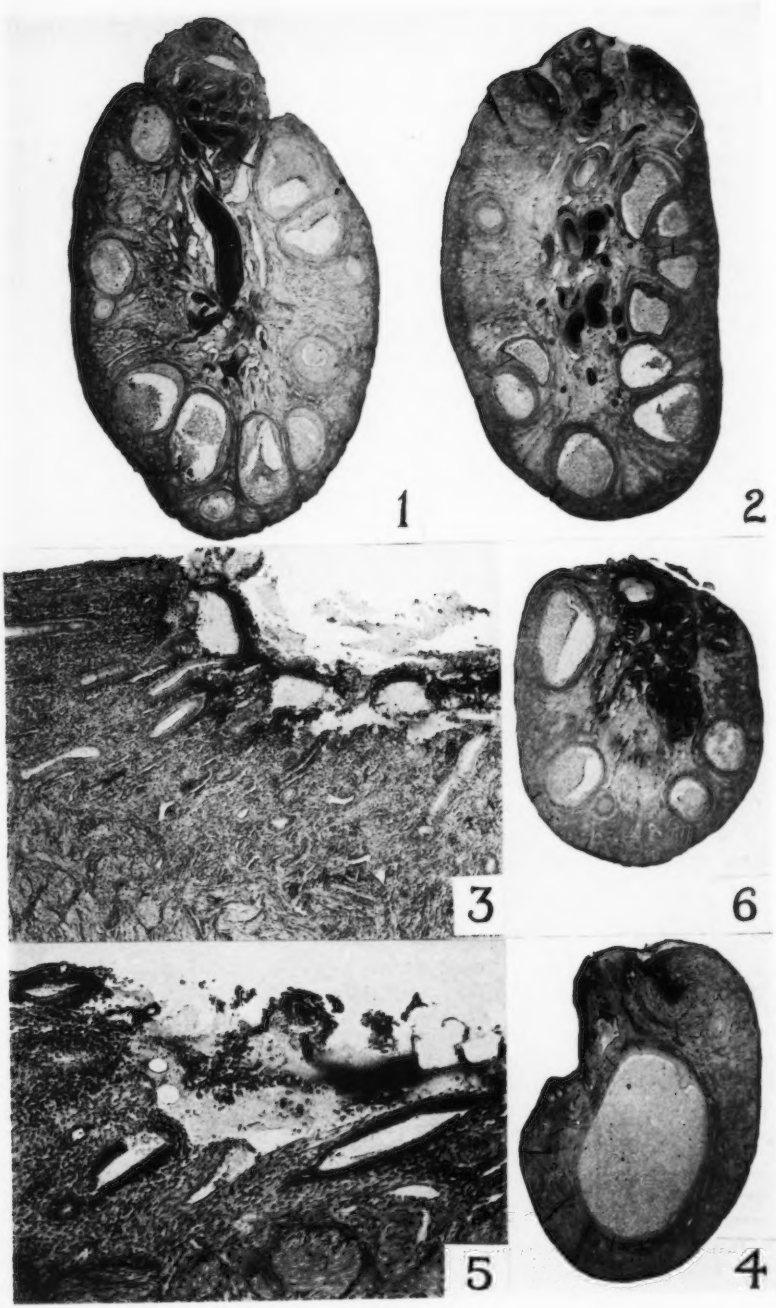
Figs. 1 and 2. Left and right ovary of no. 9, ×11, fourth day of cycle, showing largest follicles in both glands.

Fig. 3. Menstruating endometrium of animal 9, ×45. This was the second day of bleeding, five days after ovariectomy and nine days after the first day of the preceding menstruation.

Fig. 4. Left ovary of no. 2, ×11, fifth day of cycle, showing largest follicle found in the ovaries removed before the thirteenth day.

Fig. 5. Menstruating uterus from no. 2, ×94 on the fifth day of bleeding, which commenced eleven days after the first day of the preceding menstruation.

Fig. 6. Left ovary of no. 12, ×11. The removal of this single ovary resulted in menstruation four days later.



Figs. 1–6

endometrium after bilateral ovariectomy. No. 9 was autopsied on the second day of menstruation and the interval between the end of the last normal menstruation and the beginning of the post-operative menstruation shown here was only five days. Animal 2 (fig. 5) was autopsied on the fifth day of the induced bleeding. Destruction of the mucosa was still in progress from an endometrium which was inactive for only eight days between menstruations. Corner (1927) and Hartman (1927, 1928) have established the occurrence of menstruation without ovulation and the premenstrual changes of the endometrium in the monkey. A similar condition is found in the sections presented here. The straight uterine glands and the low endometrium identify the organs as interval in type.

SUMMARY

1. In the Pithecus (Macacus) rhesus, it has been found that excision of both ovaries early in the cycle (from 72 hours to the tenth day and one instance on the thirteenth day) was followed by menstruation after an interval of five or six days.

2. In amount and duration the saguineous discharge was not distinguishable from the normal menses of the individual animal.

3. Histological examination of the ovaries showed that bleeding could occur in the absence of a mature follicle or young corpus luteum.

4. Menstruation from an endometrium of interval type occurred in animals operated upon on the fourth and fifth days of the cycle.

5. Removal of a single ovary on the fourth day of the cycle was followed in one case by menstruation on the fourth day following the operation but in another animal no post-operative menstruation occurred.

6. Both bilateral and unilateral ovariectomy failed to induce bleeding when performed on the first day of menstruation.

BIBLIOGRAPHY

ALLEN, E. 1927. Carnegie Contrib. to Embryol., xix, 1.
1928. This Journal, lxxxv, 471.
COERNER, G. W. 1927. Journ. Amer. Med. Assoc., lxxxix, 1838.
1923. Carnegie. Contrib. to Embryol., Pub. No. 332, 75.
FRAENKEL, VON L. 1903. Arch. f. Gynaek. (Berlin), lxviii, 438.
HALBAN, J. AND R. KOEHLER. 1914. Arch. f. Gynaek. (Berlin), ciii, 575.
HARTMAN, C. G. 1928. Anat. Rec., xxxviii, 47.
1927. Anat. Rec., xxxv, 13.
1931. Journ. Mammology, xii, 129.
HARTMAN, C. G., W. M. FIROR AND E. M. K. GEILING. 1930. Proc. Exp. Biol. and Med., xxviii, 185.
HEAPE, W. 1897. Phil. Trans. Roy. Soc., 188B, 135.
OSTRCIL, A. 1924. Casop. lek. cesk. (Praze), lxiii, 139.
PRATT, J. P. 1927. Endocrinol., xi, 195.
SEITZ, L. AND H. WINTZ. 1919. Monatschr. f. Geb. u. Gynaek. (Berlin), xlix, 1.
VAN HERWERDEN, M. A. 1906. Monatschr. f. Geb. und Gynaek. (Berlin), xxiv, 730.
ZUCKERMAN, S. 1930. Proc. Zool. Soc., xlv, 45.